IRON METABOLISM

IRON METABOLISM
AND ITS CLINICAL SIGNIFICANCE

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PREFACE TO THE ORIGINAL EDITION

THIS monograph represents the results of our investigations in the domain of Iron Metabolism, a work which we have been systematically pursuing for several years.

Proceeding from the study of the Porphyrins, we deemed it necessary to follow accurately the course of the iron which is often bound up with the Porphyrin ring. As a result we have approached more closely to the problem, so vital both from a clinical and general biological point of view, of the blood and tissue haemins, that is, of the blood and tissue pigments.

In accordance with our initial concept we have endeavoured to view the serum iron not only as a factor in the determination of certain definite clinical problems, but rather as the manifestation of a biologically important element in connection with the function and regulation of the total organism.

Scientific discoveries are often the result of co-operation, and it has been our good fortune to have been brought into touch with very many colleagues and collaborators who have facilitated our work. To all of them we wish to express our grateful thanks, above all to Dr. *J. J. Schenk* (Chemistry) who furnished us with the iron determinations and with the methods of investigation which had been elaborated for that purpose.

The following work was carried out with the support of the J. Macy Jr. Foundation of New York, and its publication was made possible through the kindness of the firm of Benno Schwabe and Co. of Bâle. To them we here wish to express our particular thanks.

A. VANNOTTI and A. DELACHAUX

Lausanne, March 1st, 1942

PREFACE TO THE ENGLISH TRANSLATION

SINCE the completion of the German edition of this book, published by Messrs. Benno Schwabe and Co. of Bâle in 1942, the problem of the biological and therapeutic significance of iron has been further extended. This development has been particularly fostered by the introduction of radioactive isotopes in the study of iron metabolism and by the enrichment of our therapeutic agents by parenteral divalent and trivalent iron preparations. In this domain comprehensive and valuable contributions have been furnished by British and American authors in recent years, and it is for that reason that I have welcomed Dr. E. Pulay's suggestion that an English translation of the book should be made, hoping by this means to be able to establish closer contact with all scientists of the English-speaking world who are interested in the problem of iron metabolism.

For this translation several chapters have been recast and supplemented and I have added, for the benefit of the practising physician, a Third Part in which I have endeavoured briefly to summarise the experiences gained by the clinical study of iron metabolism in serving the purposes of practical iron therapy.

It is my pleasant duty in this connection to express my profound appreciation to Dr. Erwin Pulay and Miss Beatrice Cunradi of London for the great pains they have taken in preparing this excellent and accurate translation of the book.

I continue to be greatly indebted to the Josiah Macy Jr. Foundation of New York. Thanks to its generous support I have been enabled to carry out the investigations on iron which I started before the war. The present English translation will serve to draw attention to the valuable work of this Foundation in the field of Medicine.

Finally, I wish to express my sincere thanks to the publishers for the excellent publication of this translation and for the trouble they have taken in the printing of this edition.

A. VANNOTTI

Lausanne, July 1948

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EVEN the ancients believed that iron played an important role in human pathology. The Egyptians, and after them the Greeks and Romans with *Galen* and *Pliny*, and later *Paracelsus* in the Middle Ages, all attributed great therapeutic properties to iron, and its use as a strengthening agent was universally recognised.

Thus, from the earliest times, iron has been an important therapeutic factor and the subject of profound medical interest. But the part played by iron in the domain of biology was not known until much later, notably after *Lemmery* and *Geoffroy* discovered in 1713 that iron was present in animal tissues. Shortly afterwards *Badia* demonstrated its presence in blood—a discovery confirmed a hundred years later by *Albrecht von Haller*. Since that time great emphasis has been laid on the importance of iron, particularly in connection with blood formation, and especially with haemoglobin formation. Hence iron has gradually assumed a position of paramount importance in the treatment of blood diseases, especially the various forms of secondary anaemia.

These facts have long constituted the basis of our knowledge and of our biological and medical studies. But in recent years numerous clinical and experimental observations have shown, not only that the presence of iron in the organism is important for cell formation and for the part played by haemoglobin in the organism, but that iron has much more far-reaching activity. Actually iron plays the part of catalyst in a number of chemical processes, and so this led gradually to the conviction that this metal is not only useful, but even indispensable for the life of animal cells and of the entire organism.

The studies which have been based on this new conception of the biological function of iron are still far from having reached a final conclusion; they have the merit, however, of having led to a number of new problems and theories. In biochemistry many facts have been definitely established which have directly stimulated the clinical outlook of human pathology. Although much is still unknown, we consider it highly important that the attention of physicians and biologists be directed to a series of symptoms and processes which in our opinion are closely interwoven with iron metabolism. We therefore intend in this book to show the relation between certain chemical processes which need the presence of iron and the clinical manifestations which

depend upon them. Finally, we shall endeavour to show that the study of iron metabolism, even outside the domain of erythropoiesis, is to-day indispensable for every practitioner, from the point of view both of diagnosis and treatment.

PART ONE

THE BIOLOGICAL SIGNIFICANCE AND NORMAL METABOLISM OF IRON

I. General Biological Considerations regarding the Physico-chemical Properties of Iron

Before proceeding to the exact study of iron metabolism, we wish to mention some purely physico-chemical properties which serve to clarify the significance of iron in cell organisation. Very extensive studies, for instance those of *Starkenstein* and his school, are of great value in this connection: the importance of iron in animal life is particularly associated with its catalytic properties, that is to say, with its capability of partaking either directly or indirectly in chemical phenomena and of liberating certain processes which could not operate in the absence of iron. The conclusions reached by *Jakusizi* as a result of his investigations clearly indicate the biological importance of this metal. This author has shown that the iron content of blood increases in proportion to the height attained in the animal scale. *Kamegal* for his part has found a lower iron content in the blood of invertebrates than of vertebrates.

The biocatalytic property of iron resides mainly in the attribute of varying its valency in certain of its salts according to whether they are oxidised or reduced. By reduction trivalent iron becomes converted into divalent iron, although through the operation of an oxidising agent it can be reconverted into a trivalent salt. Hence, as emphasised by *Starkenstein*, it is the power of effecting a change of valency through oxidation or reduction that constitutes the functional significance of iron and, generally speaking, of all metals in the field of biology. As a matter of fact, in addition to iron there are a number of other elements, such as aluminium, copper, manganese, all of which are capable of exerting a certain catalytic function. But in biology a process of oxidation, and conversely a process of reduction, must be based upon a simple and rapid mechanism. Nevertheless, in the case of most metals this conversion is rather slow and complicated; and this is why iron alone possesses real biocatalytic value. Moreover iron does not cause a fundamental change in the environment in which the catalysis develops, nor in the substances which are directly involved in or associated with the catalytic process. This second quality is an indispensable biological factor, since the reactions nearly always occur in the

presence of proteins which, under other conditions, would easily be denatured or precipitated.

In examining the properties which are responsible for the biocatalytic activities of iron, *Starkenstein* stresses the fact that the co-operation with the living substance does not depend upon the quantity of the metal involved but upon the interaction of physico-chemical properties, which can be summarised as follows: solubility in water, formation of iron-protein complexes of a colloidal nature without flocculation, great oxidation-reduction power.

Suffice it to remark at this point that, chemically considered, biological iron is usually found in the form of a divalent or trivalent salt, and that the monovalent, hexavalent or octovalent combinations are of no great biological interest. The degree of oxidation and the rapidity of reaction with oxygen are in part dependent upon the anion which accompanies the iron. According to *Simon* and *Kötschau* iron bicarbonate is the iron salt which is most sensitive to oxygen. The speed of reaction is not only dependent upon the anion, but upon a number of additional factors, among which light plays some part. As a matter of fact red light and ultra-violet and X-rays may serve to accelerate the oxidation of the iron salts. This phenomenon has been emphasised by various authors. We mention in this connection the observation of *Vannotti* regarding the decomposition of myoglobin under the influence of ultra-violet light in the presence of dilute hydrochloric acid. This author was able to demonstrate that if a section of muscle rich in myoglobin was placed in a hydrochloric acid medium and subjected to intensive ultra-violet irradiation the myoglobin rapidly became converted into porphyrin. Thus the ultra-violet rays are able indirectly to liberate the iron from the haemin ring. Several organic iron salts are reduced by light, in which case the anion acts as the reducing agent.

In addition to its favourable biological influence, iron may, under certain conditions, exercise an injurious effect upon certain physiological processes; for instance, it may facilitate protein denaturation, certain processes of agglutination, and haemolysis.

II. The Metabolism of Iron

Before approaching the problems associated with the activity of non-haemoglobin iron in the organism, it is essential to become acquainted with the physiological metabolism of this metal, that is, the processes connected with its absorption, distribution, storage, conversion within the organism, and finally with its excretion.

(*a*) *The Absorption of Iron from the Intestine*

Small quantities of organic iron salts and compounds are introduced into the intestinal tract with the food. In the stomach part of this iron is freed by HCl and is ionised in its reduced form. This fact renders intelligible the assumption that part of the iron is absorbed in the stomach. The gastric wall, which has powerful negative charges, facilitates the absorption of the positively charged iron, and according to *A. Jung* it permits the nutritive salt to penetrate directly into the blood. But most of the iron absorption appears to take place in the small intestine, particularly at the level of the duodenum. Thus *Starkenstein* obtained maximal utilisation of the iron complexes when these were administered *per os*, with the addition of fats—a fact which would indicate that duodenal absorption is effected with the help of bile. From this it can be inferred that most of the iron penetrates the intestinal wall where there is most intensive absorption, in a part of the intestine not too remote from the outlet of the stomach. There the iron is found in a simple form, not yet incorporated in an organic complex, as is the case after prolonged contact with the contents of the intestine.

Here it should be emphasised that the duodenum is not necessarily the exclusive site of iron absorption; other sections of the small intestine may also exercise this function, as can clearly be observed in cases of stomach resection, and even of gastro-enterostomy.

The serum iron following the ingestion of iron in a case of stomach resection indicates a distinct rise of the serum iron a few hours after iron administration.

	On empty stomach	Hours after peroral administration 2	4	6
Serum Iron	88 γ%	130 γ%	122 γ%	92 γ%

It is not the special anatomical structure of the intestinal wall, but the fact that the iron has only recently left the cavity of the stomach, that determines the site of iron absorption. Under normal conditions this occurs in the duodenum.

Only a small portion of dietary iron is absorbed through the intestinal tract. As a general rule this quantity is but slightly

affected by a heavy oral intake, although great differences are noted in accordance with the various iron salts used. Some of these pass very quickly through the intestinal wall, others very slowly and only in small fractions, whilst others have to be reconverted in the intestinal canal before they can be absorbed. The admirable work of *Starkenstein* and his pupils demonstrates that the ferrous salts are most easily absorbed through the stomach wall, in contrast to the ferric salts, which have probably to be converted into ferrous salts before they are able to pass through the intestinal wall. This process of reduction can only take place in the stomach, and thus it depends to a great extent upon the quantity of HCl there present and upon the length of time that it is in contact with this acid. Moreover, only a part of the ferric salts can be reduced, a fact which explains *the great difference between the conditions of absorption of ferrous and ferric salts.* (The dog absorbs both forms well.)

Lederer assumes, on the basis of a series of experiments, that in addition to the HCl the gastric juice also contains a ferment which has the power of liberating the iron in an available form from its organic combination, and thereby of effecting a combination with hydrochloric acid. In the absence of this catalyst, which however must not be confused with the endogenous anti-pernicious factor (intrinsic factor), the liberation of the nitritive iron, even in the presence of free HCl, is impaired. In the opinion of *Heilmeyer*, it is not the hydrochloric acid, as is generally assumed, which is the most important element in iron absorption, but rather Vitamin C. This would explain the rapid curing of anaemia in infants nourished on cow's milk when this was replaced by human milk. Both foods have an equivalent iron content, but human milk is distinctly richer in Vitamin C. In this connection there would appear to be interest in the findings of *Luksch* and *Rominger*, who attribute to Vitamin C a favourable influence on iron absorption in the digestive tract. According to these authors Vitamin C stimulates secretion of gastric juice, thus indirectly facilitating iron absorption from the intestine. Finally, it should be mentioned that *by virtue of its reducing power Vitamin C might protect the reduced iron from oxidation in the digestive tract, thus serving as an important factor in promoting iron absorption.*

The iron salts which are most easily ionisable are best absorbed by the organism. From a pharmacological point of view the ferrous salts are much more toxic than the ferric salts, a fact doubtless attributable to their easier and quicker absorption. But among the ferrous salts there are also found distinct differences in absorption, as is shown by the following figures of absorption for the same quantities of iron and the same individuals:

	0	2 hrs. later	4 hrs. later	6 hrs later
	γ%	γ%	γ%	γ%
Iron chloride (ferric) ..	30	85	146	150
Ferrum reductum	40	148	128	100
Iron chloride (ferrous) ..	25	170	188	85
Iron lactobionate (ferrous) + calcium lactobionate ..	40	185	320	204
Iron formate (ferrous) ..	30	112	275	250
Iron sulphate (ferrous) + ascorbic acid	48	195	300	163

The cause of this is that the ferrous salts are in part rapidly oxidised in the intestine and hence less perfectly absorbed. If however the iron is stabilised in its reduced form by the addition of both ascorbic acid and calcium lactobionate its absorption as a ferrous salt is assured and the blood iron level is accordingly higher, indicating better penetration of the intestinal wall.

Recently *Vannotti* and *Kalbermatten* made interesting observations on the peroral absorption of iron under the influence of para-aminobenzoic acid. As a result of the systematic administration of para-aminobenzoic acid these authors showed that in the severe forms of iron-deficiency anaemia (above all in the essential form) there was a certain lack of this acid. On the basis of these investigations *Vannotti* and *Kalbermatten* undertook an experimental study of the functional relations between iron metabolism and para-aminobenzoic acid and determined that if the patients were previously treated with fairly large doses of para-aminobenzoic acid the blood-iron curve after peroral administration was flatter if ferric salts or iron in a non-stabilised reduced form were given. On the other hand, the blood-iron curve is higher after pretreatment with para-aminobenzoic acid, if the iron is stabilised in its reduced form. These authors explain this phenomenon by the fact that the para-aminobenzoic acid forms with the oxidised iron complexes which are not easily absorbed.

But these complexes are not formed if the iron is present in a stable reduced form.

However, once the iron has passed through the intestinal wall and has been converted into the oxidised form in the blood stream the iron–para-aminobenzoic acid complex can be formed. This has a special affinity for the cell membrane and can hence easily associate itself with the erythrocytes and the tissues. Actually, the iron level in the erythrocytes shows a marked increase after the addition of para-aminobenzoic acid.

After the iron has penetrated the intestinal wall it forms complexes and combines with the blood proteins; hence it is never found in the blood in an ionised form. The salts with large molecules practically never penetrate the intestinal wall, as *Lintzel*

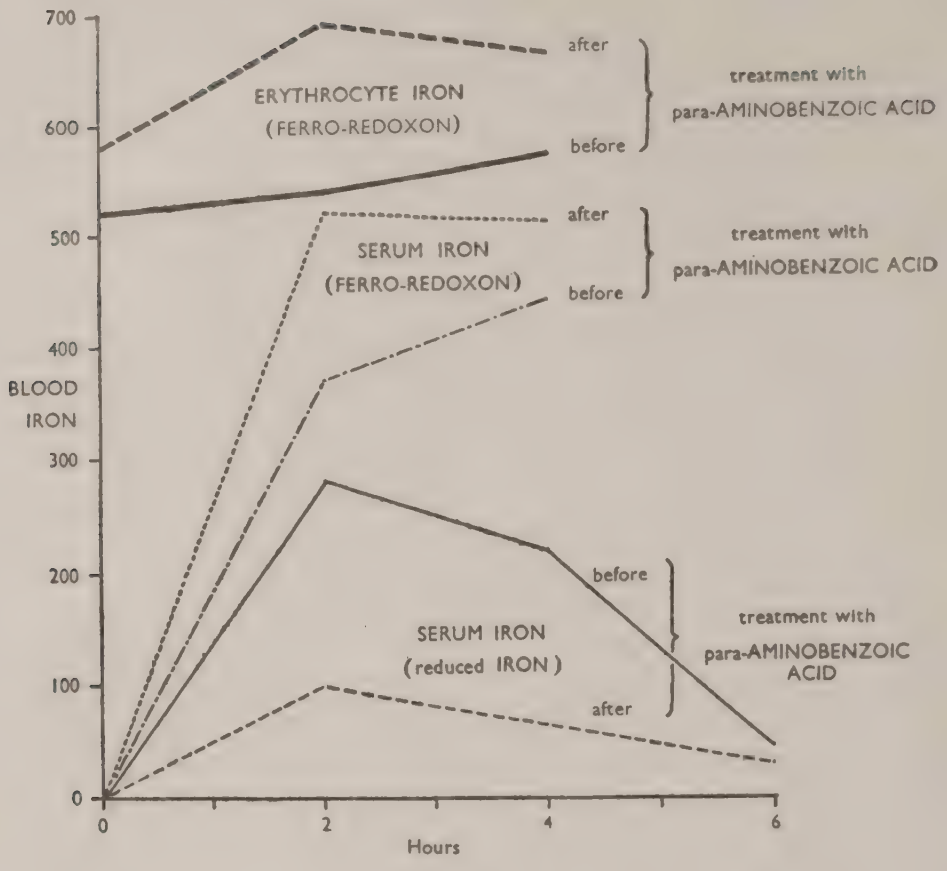


DIAGRAM 1

Influence of para-aminobenzoic acid on the distribution of iron in blood serum and erythrocytes.

has shown in the case of haemoglobin and yolk of egg. The organic iron complexes, such as haematin and haemoglobin, those of the vegetable kingdom, etc., are therefore of little nutritive significance as iron carriers—indeed, of practically none at all. As a rule their iron is not absorbed by the intestine. *Barkan* and *Lintzel* measured quantitatively the iron liberated from the haemoglobin through HCl, pepsin and pancreatin, but only succeeded in releasing from 5 to 8 per cent of the haemoglobin iron. This finding is of great interest, in that it proves that the gastric juice lacks the power of liberating iron and thereby decomposing the haemoglobin. Various authors have studied the problem of haemoglobin decomposition in the intestinal canal (*Boas, Fischer, Schumm, Snapper, Papendick, Haurowitz*). *Haurowitz* found that after taking 50 cc. of blood, 85 to 90 per cent of the blood pigment was converted into protohaemin in the intestine, 5 to 8 per cent into deuterohaemin, only 2 to 3 per cent into protoporphyrin, and 0.5 to 1 per cent into deuteroporphyrin. The deuterohaemin, as also the deuteroporphyrin and the protoporphyrin, are products

arising from the action of the intestinal flora on the blood pigment; thus they are not produced by digestive enzyme action. This fact is important for it confirms the above-mentioned experiments *in vitro* performed by *Barkan* and *Lintzel*. Furthermore it confirms the observations of *Vannotti* and others, who consider the formation of porphyrin in the intestinal canal to be a sign of excessive putrefaction.

The amount of iron absorbed is not proportional to the amount of iron administered *per os*; it largely depends on the organism's need for iron, as is shown in the balance tests on human subjects performed by *Reinmann, Fritsch* and *Schick*, and by *McCance* and *Widdowson*. *Whipple* and his followers came to the same conclusion, namely, that in experimental chronic haemorrhagic anaemia a dog suffering from iron deficiency absorbs up to 9 per cent of the iron administered *per os*, whilst controls absorb only 1 per cent. Thus it is seen that iron enters into the circulation only in cases of need. The capacity of storage organs to take up additional iron appears to be an important factor in connection with the quantity of the metal to be absorbed. The American authors agree that the small intestine possesses the capacity of absorbing iron and believe that the mechanism for regulating the quantity absorbed is probably located at the level of the mucous membrane and is possibly influenced by the organism's degree of anaemia and lack of iron. According to *Fowler* and *Barer* the smallest quantity of nutritive iron needed by the human body for the maintenance of the iron equilibrium is from 12 to 15 mg. daily. However, this quantity varies in accordance with the degree of acidity of the gastric juice and the periodic losses of blood and iron.

On the whole, these observations can be confirmed in human subjects, as is seen by the following values of the serum iron capable of being split off, in a normal person and in one suffering from iron deficiency, after oral iron administration (1 g. ferri reducti).

	Before administration	2 hrs. later	4 hrs. later	6 hrs. later
	γ%	γ%	γ%	γ%
Iron-deficient individual ..	52	170	365	208
Normal individual ..	121	132	126	114

The quantities of iron absorbed appear under normal conditions to be dependent upon the iron needs of the organism.

In connection with a technique subsequently to be described we investigated in what form the freshly absorbed iron is found in the serum. For this purpose the serum iron of a normal person was determined fasting, and again three hours after oral administration of 1 g. of reduced iron. It was found that after three hours the lightly bound iron fraction had increased from 1 to 5,

and the strongly bound iron fraction from 1 to 1.5, whilst there was only a slightly perceptible increase of the iron that could not be split off. The last-named increase was probably brought about by the conversion of a small portion of the separable iron into the non-separable forms in the course of the first three hours.

Recently *Granick* established the fact that when guinea pigs were fed with divalent iron a definite increase of the ferritin content of the mucosa of the small intestine could be observed. This led him to assume that there exists in the intestinal mucosa an equal balance of divalent and trivalent iron (in the form of ferritin). A reduction of the blood iron level causes the removal of divalent iron from the cells of the mucosa, and this accordingly results in a diminution of the ferritin iron. If this reduction is so considerable that the cell is no longer in a condition of physiological saturation iron is again absorbed from the small intestine.

(*b*) *The Distribution of Iron in the Organism; the Conversion and Storage of Iron in the Organs*

It is the special merit of *Starkenstein* to have studied and described iron conversion in normal individuals. According to this author the ferrous salts, once they have been absorbed, circulate for a varying length of time in the blood before being deposited in the organs. *Strangely enough, the spleen, which is known to be an organ of iron storage, is unable to fix the ferrous salts which circulate in the blood. It retains only the oxidised iron, i.e. the ferric salts. Gradually the circulating ferrous salts become oxidised; they become converted into ferric salts and lose their quality of "active iron"* (*Starkenstein*). *Actually, in the domain of biology, it is the reduced iron that plays the part of the catalyst; as soon as it is oxidised it forfeits every catalytic property and ceases to take an active part in the biochemical processes.*

Recently *G. H. Whipple* and collaborators, in particular, have studied iron metabolism with the radio-active iron isotopes (an iron form characterised by its radio-activity, whereby it is easily distinguished from the rest of the iron). By means of this method of investigation these authors followed the migration of the iron introduced into the animal organism *per os* and obtained similar results to *Starkenstein*. Iron is transported from the intestinal tract to the various centres of consumption in the first instance in the blood plasma; but a few hours after its absorption the greater part of this iron has already reached the red blood corpuscles, which thus appear to have a share in the transport of iron. The liver and bone-marrow rapidly fix the freshly absorbed iron, but this does not, however, appear to be true of the spleen.

The problem of the utilisation of the iron after intestinal absorption can also be studied by following up the blood-iron level after intravenous iron injection. *Starkenstein* found that iron chloride (ferrous) administered intravenously is rapidly oxidised in the blood, producing a ferri-globulin complex. He believes that the iron of this complex is not reduced until a later stage, when it would either partake in the building of haemoglobin or serve as deposit iron.

The oxidised iron salts, on the other hand, would speedily be transported to the organs of excretion or storage. *Lederer* has shown that the intravenous administration of ferric salts can also be successfully used to combat anaemia. This fact would therefore indicate that even iron in the oxidised form can be utilised for the formation of blood pigment.

More recently *Vannotti* was able to demonstrate that the intravenous administration of iron salts in the reduced form has particularly favourable therapeutic results in tissue-iron deficiency, but is less effective for haemoglobin synthesis; furthermore, that after intravenous injection the blood-iron curves in the same subject vary in accorance with the form of iron used.

The following diagram serves as an example of this:

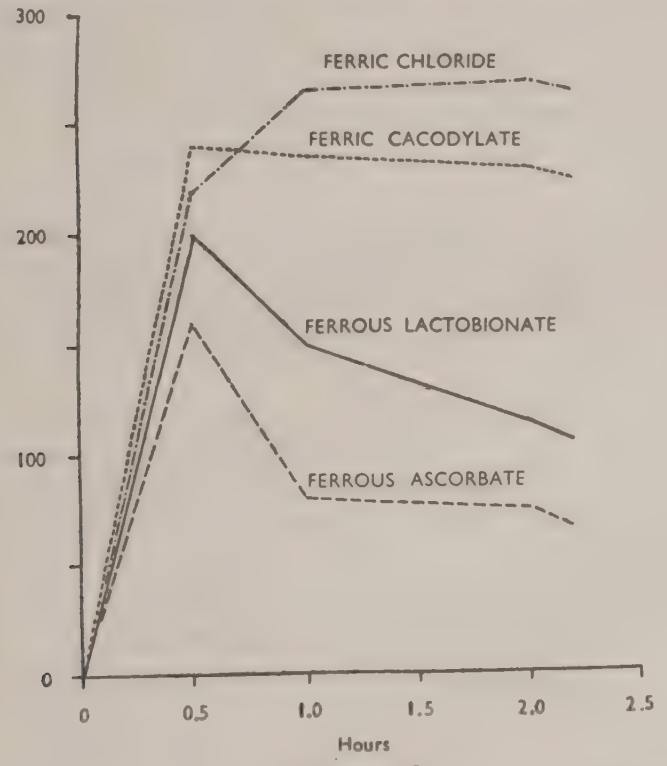


DIAGRAM 2

Variation of serum iron after intravenous injection of 6 mg. of iron in the form of ferrous ascorbate, ferrous lactobionate, ferric cacodylate and ferric chloride.

The curves of the trivalent iron preparations (oxidised form) are very high and slowly decline to the initial values. The curves of the divalent iron preparations (reduced form), on the other hand, are less high and rapidly sink to lower values. This fact explains why the reduced iron preparations disappear much more quickly from the circulation; in other words, why they are much more quickly used up by the tissues than are the oxidised iron preparations.

The investigations of *Mamie*, who determined the iron content in the organs of rabbits after intravenous Fe administration, clearly reveal the difference between the ferrous and the ferric salts.

According to *Mamie's* experiments on healthy rabbits and on others rendered anaemic, the organs showed after iron administration the following differences in their content of easily split-off iron:

The muscle of the healthy animal showed no increase of iron, that of the anaemic animal disclosed a distinct reduction of the iron content, which after intravenous injection of iron in the reduced form returned to normal. (But the administration of iron in the oxidised form had no effect.) The muscle treated with iron showed a marked increase of its cytochrome and myoglobin content.

In anaemia the iron content of the liver, spleen and kidney decreases conspicuously. However, this reduction is speedily remedied by intravenous injections of iron. This is seen particularly in the liver after administration of ferrous salts, and in the spleen after ferric salts.

The bone-marrow is especially rich in iron, although in anaemia it loses much of it. This iron deficiency is quickly restored after intravenous injections of iron, and the improvement of the blood picture is much more evident after treatment with ferric salts than with ferrous salts.

These findings have led us to assume that the ferrous salts (reduced iron) have a selective effect on the tissues, above all on the striated musculature, in the form of cell catalysts, and a rather feeble effect on blood pigment formation. The ferric salts (oxidised iron) show no biocatalytic activity at the level of the tissues. After they have circulated in the blood stream for a longer period they are gradually deposited in the spleen and in the organs of deposit, where they are able to exercise a more pronounced influence on haemoglobin formation than can the ferrous salts.

The spleen, the organ of deposit of oxidised iron, mobilises its reserves once the supply of active iron available in the organism, that is, the ferric salts, is exhausted, or whenever the body needs

iron for regeneration. The ferric salts, for their part, can be reduced to inactive ferrous salts by the reticuloendothelial system. This reduction takes place in the liver where the inactive ferrous salts are also stored. Here they remain in case they should be needed for future haemoglobin formation.

It has been stated above that the presence of iron in the tissues has long been known. This iron does not depend on the presence of haemoglobin alone. Actually the quantitative determinations of iron in the tissues show higher values than can be accounted for by the existing blood pigment. This observation was made by various authors, starting with *Abderhalden*. In 1877 *Quincke* had already noted the presence of iron in the tissues; he spoke of a "physiological siderosis", and particularly of a siderosis of the erythropoietic organs.

Next to haemoglobin, haemosiderin is one of the commonest forms of iron in the tissues. Its name was given by *Neumann* (1888). After extensive cellular disturbance there is an accumulation of haemosiderin in the liver. It may also be found in old haemotomata. Haemosiderin does not possess an exact chemical constitution; the iron here appears in an oxidised form difficult to reduce. According to *Abderhalden* haemosiderin consists merely of organic substances impregnated with Fe_2O_3.

Recently the constitution of haemosiderin appears to have been defined exactly. *Granik*, *Michaelis* and collaborators, as well as *Fankuchen*, established the fact that the organs in which haemosiderin collects contain a protein with a molecular weight of approximately 500. This protein can be directly purified through crystallisation as a cadmium salt and it possesses properties which facilitate the collection of enormous quantities of colloidal ferric hydroxide in its crystal meshes. Haematoidin, a pigment also found in old haematomata, contains no iron at all. *Fischer* considers it to be identical with mesobilirubin. In addition to the iron forms which can easily be detected in histological sections, a number of iron-containing substances enter into the composition of cells. The nucleoproteins, i.e., the protein-containing substances which form the cellular nucleus, contain small quantities of iron (*Halliburton* and others). *Myerhof* and *Lohmann* have described the occurrence of iron in the proteins of the muscle; here it takes the form of an iron pyrophosphate. Even the lipoids may be bound to iron, as for instance certain lipoid complexes of the spleen (*Burrow*). Finally, chemical examination shows that all tissues, even after they have had all traces of blood removed, contain small quantities of iron originating from other cellular elements. Actually the tissues contain an iron fraction which can be extracted with diluted hydrochloric acid (*Starkenstein* and

Weden), and which is found chiefly in the liver and spleen. In addition there is yet another group of iron-containing cell components which play a special role; this comprises the ferments whose catalytic effect is largely associated with the presence of this metal. In view of their biological and clinical significance these ferments will be considered in a special section.

In iron metabolism an important part is played by the reticulo-endothelial system. The phagocytosis of the blood corpuscles and the considerable role assumed by the reticulum in bilirubin formation constitute sufficient proof of the importance of this system in the conversion of iron. The regulation of iron metabolism can be noted particularly in the reticulum of the liver and in the bone-marrow through the maintenance of a certain degree of equilibrium between destruction and synthesis of the blood pigment. It is to *Eppinger* that we are indebted for exact information regarding this co-operation between the hepatic reticulum, the centre of haemoglobin break-down, and the bone-marrow reticulum—an important organ of blood pigment synthesis.

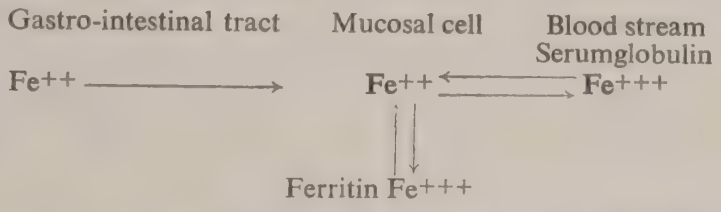
This author also formulated the interesting theory that in aplastic anaemia and in polycythemia the function of the reticulo-endothelial system is diminished; in one case there is diminished functioning of the bone-marrow, in the other of the liver. Finally, he holds that in haemochromatosis the reticulum has lost the power of restoring the phagocytosed iron to the circulation—a fact which might account for the excessive quantities of iron found in the tissues. We shall revert to this problem at a later stage, but the theory of *Eppinger* clearly demonstrates the importance of the reticulo-endothelium, considered as a uniform system, and its close relation to iron conversion. In conclusion it should be remarked that only colloidal iron is phagocytosed by the cells of the reticulo-endothelium.

The role of the spleen in iron metabolism has been considered in a number of writings, of which only a few can be mentioned. *Pana* observed after splenectomy a temporary increase of the iron content of the liver. This observation has been confirmed by some authors and rejected by others. Here we will merely briefly mention the various works of *Asher* and his school, and finally those of *Lauda*. *Asher* reached the conclusion that the spleen regulates blood metabolism in such a way as to permit the iron which has become useless to the organism to resume, when necessary, any one of its biological functions. *Lauda* does not support Asher's views regarding the significance of the spleen in iron metabolism; he holds that the present extent of our knowledge does not justify our forming any opinion regarding this matter. Various other authors,

moreover, refuse to attribute to the spleen any influence whatever on iron metabolism.

It is important to know in which form iron is deposited in the organs and chiefly in the liver and spleen. From the works of *Granick* and his collaborators, we know that ferritin assumes the function of iron storage in the organs. Ferritin is also found in the duodenal mucosa. When ferrous iron is fed, ferritin increases (4–7 hours after feeding); it is concerned with the regulation of iron absorption. Granick showed that the feeding of iron leads to an increase in the concentration of the specific protein apoferritin.

In this case, the excess of iron absorbed is converted to the ferric state and is stored temporarily in the form of ferritin in the mucosa. By saturation of the mucosa with iron, the production of ferritin and also iron absorption are stopped (block of the mucosa).



Ferritin is also found in high concentration in the red bone-marrow (iron storage for blood formation). Extensive bleeding lowers the ferritin and apoferritin content of the spleen (probably reutilisation for blood production).

Mamie and *Kalbermatten* studied, in our laboratories, the distribution of the iron injected intravenously in the rabbit, both in a normal state and in chronic anaemia.

In the anaemic rabbits these authors found, upon comparing the values obtained with those of the healthy animals, a marked diminution of iron in all the tissues. The iron contained in the peripheral tissues, as well as in the organs of storage, was mobilised and either lost through bleeding, or utilised immediately for the new formation of haemoglobin. This fact is particularly striking in the case of the spleen, since its easily split-off iron can by this means be again restored to the circulation and utilised for haematopoiesis. Thus it is seen that in severe cases of anaemia the spleen also contains some iron that can be mobilised, as is indicated by a reduced level of this iron fraction.

In the muscles the diminution of the iron level is also very marked; after a removal of 200 cc. of blood there remains only one fifth of the previous quantity of iron. This proves that for the regeneration of the blood the organism utilises not only the iron of the organs of storage, but also that of all the tissues; furthermore.

that haemorrhage may involve a loss of peripheral iron—a condition which rapidly leads to tissue anaemia due to lack of iron. This is clinically manifested by abnormal fatigue and adynamia, by various trophic disturbances, and in the young by disturbances of growth, all the more so since it concerns a biologically active form of iron which by virtue of its catalytic property participates in the various mechanisms of cellular respiration.

Parenteral injections of iron in the animals of the first series (non-anaemic) provoked a marked rise of the level of separable iron in all the organs, above all in the liver, the spleen and the bone-marrow, whilst in the muscles and kidneys there were few changes. Consequently, as the non-anaemic organism is not in need of any supplementary iron, it is able to deposit this in its storage organs. This increase is more marked in the case of Sandoz iron than in that of ascorbic acid iron, probably because of its very great stability, which permits it to remain active for a longer time.

In the anaemic animals the injections of iron led to a distinct increase of the iron content in all the tissues, above all at the level of the bone-marrow and the muscles. This was less obvious in the liver and still less in the spleen and kidneys. Hence it is seen that the iron is transferred in the first instance to the tissues possessing great biological activity, with the result that the tissue anaemia, both at the periphery and at the level of the bone-marrow, is largely compensated for by increased haematopoiesis. Only at a later stage do the deposit organs supplement their store of iron.

In acute anaemia the injection of iron also provokes interesting modifications. The content of biologically active iron is higher in all the organs after six hours than after twenty-four hours, whilst that of the serum iron is 170 $\gamma\%$ and 80 $\gamma\%$ respectively. The different response of the spleen where the iron level is higher after twenty-four hours than after six hours indicates the important role played by this organ as a deposit of iron after it has been in circulation for twenty-four hours, during which time this metal has been able to develop its biocatalytic activity at the periphery. The iron injected has been rapidly drawn to the tissues, where it is first found in its active form, whilst later a great part of it is fixed in the form of a stable complex. Nevertheless, as a result of its stabilisation, which increases its biological activity, this iron possesses a particularly marked action at the periphery, where it is able to exercise its catalytic function and finally to participate in the formation of the stable complexes of the cellular haemins. The same mechanism is found at the level of the haematopoietic organs, the activity of which is stimulated before the complexes are incorporated in the porphyrin ring for the formation of haemoglobin.

Lastly, it is necessary to report the quantitative estimation of iron contained in the body.

Hahn, Whipple and their collaborators found a total quantity of 4.30 g. iron in the human adult organism (blood haemoglobin iron, 2.65 g.; available iron, 1.32 g.; unavailable iron, 1.35 g.).

In the dog, *Hahn* found: haemoglobin iron, 57 %; myoglobin iron, 7%; parenchyma iron (cellular pigments and enzymes), 16%; storage iron, 20%.

The following table shows us the average values of the iron contained in various tissues under normal conditions, in children (according to *Salvadei*) and in adult organism (dog) (according to *Bogniard, Hahn* and *Whipple*):

	Children mg.%	Adult organism mg.%
Spleen	15.5	46
Liver	11.96	25
Heart	7.63	4
Lungs	7.5	7
Marrow	—	15
Kidney	6.3	5
Brain	5.85	—
Pancreas	5.44	2
Muscle	4.62	4

(c) *The Excretion of Iron*

The iron proceeding from the various sources is distributed by the blood circulation. There it is either bound to the haemoglobin in the metabolism of which it partakes, or it exists as an indispensable element of cell breathing or of the chemical processes of the tissues, or finally as useless metabolic residue. Thus iron appears in various forms in the organism, and of this some is excreted through the kidneys, the walls of the stomach and intestines, through the biliary system, and finally through the mammary and sweat glands. In addition to these various methods of excretion there is yet another way in which the iron may leave the body, viz. through the placenta. In the latter case the iron of the mother passes into the foetus.

Before entering more deeply into the whole problem of iron excretion, we wish briefly to mention the various authors who have taken up this matter. *Widdowson* and *McCance* made a careful study of the records of four persons who, after being subjected to a diet that was poor in iron, were given strong peroral and parenteral doses of this metal. These authors were unable to observe, even during the periods of administration, any marked elimination of iron through the intestine. On the other hand, there was a slight increase of urine excretion, which is normally low.

The kidneys play a secondary part in iron excretion, and usually urine contains no iron at all. According to *Mariot* and *Wolf, Lintzel, Henriques* and *Roland,* iron excretion through the urine usually does not exceed 0.32 mg. in twenty-four hours; according to *McCance* and *Widdowson* it is not more than 0.15 mg. *Barer* and *Fowler* give the following figures after the examination of two hundred cases: in men, 0.09–1.29 mg. in twenty-four hours; in women, 0.08–1.63 mg. This excretion hardly ever changes as a result of iron intake. Even in cases of pronounced haemolysis where considerable iron excretion might have been expected the urine contained only slight traces (*Kisch*). In lead poisoning, which is often accompanied by haemosiderosis, *Lavrand* found normal iron values in the urine. It is thus seen that a large intake of iron, whether administered *per os* or parenterally, has no influence on normal kidney excretion. But, on the other hand, where there is injury of the renal parenchyma or increased capillary permeability of the glomeruli there is increased excretion. The iron is excreted through the kidney in an inorganic form, but rapidly associates itself with the colloids of the urine. Finally, mention should be made of the work of *Brabant*, who observed that, after parenteral administration to rats, the major iron excretion was effected through the kidney, particularly through the convoluted tubules, not through the glomeruli. According to this author no iron excretion is effected through the gall-bladder.

The iron content of the faeces is largely connected with the food intake. *Lintzel* believes that nearly all the iron in the stools is derived from food, that is to say, that is represents unabsorbed iron. According to this author the daily iron excretion through the intestine does not exceed 1 mg. There can be no doubt that iron excretion does take place through the intestine; the best proof of this was furnished by *M. B. Schmidt*, who found 1–2 mg. of iron in the meconium. The investigations of *Patania* have demonstrated the excretion of iron through the intestine; this occurs particularly through the colon, for he found that after the artificial excision of the large intestine of the dog, there followed decreased iron excretion through the intestine, with increased urine excretion. The systematic analyses conducted by *Schaefer* led to the same conclusions. He found that if iron absorption is chiefly effected in the small intestine, its excretion occurs principally through the large intestine. The results obtained by *McCance* and *Widdowson*, who consider that iron excretion through the intestine is slight, are confirmed by the investigations of *Maddock* and *Heath*. These authors determined the iron content in the various intestinal sections in the dog after oral and parenteral administration, as a result of which they reached

the conclusion that iron does not leave the body through the intestine.

Iron excretion through the digestive tract is not accomplished exclusively by the intestine but also, as shown by *Dhéré*, through the operation of the gastric juice; finally it is also effected by the bile. In their investigations on the dog *Henriques* and *Roland* found a daily iron content in the bile of 0.09–0.4 mg., with a total amount of bile of 20–25 cc. in twenty-four hours.

Recent American studies have determined with the aid of radio-active iron that in the dog iron excretion through the gall-bladder is on an average 0.2 mg. per day. Where there is increased haemolysis the iron excretion is higher, but with correspondingly increased excretion of bilirubin there is only 3% increased iron excretion. Intravenous injection of iron does not increase the iron excretion through the bladder (*Hawkins and Hahn*).

In post-mortem examinations *Schwartz* found 0.011–0.287 mg. of iron in the contents of the gall-bladder. More recent analyses give higher values. Thus *M. B. Schmidt* and *Starkenstein* estimate the daily iron excretion in the bile as being 1 mg.; *Eppinger* found as much as 10 mg. in human beings. The iron that is excreted in the bile is inorganic in form, but *Strausky* rejects the theory of the active participation of the bile in iron excretion, since he found no increase after iron intake. *Judd* and *Dry* similarly found that after iron intake there is no variation in the iron content of the liver and they accordingly consider that this organ is not involved in the excretion of this metal. On the other hand, *Hemmeler* stresses the importance of the liver, considering that it plays a more important role in iron excretion than does the intestine.

Finally we must emphasise the important part played by the sweat-glands in iron excretion—a factor which in the opinion of *Chevallier* must not be under-estimated.

Whipple and his collaborators, who studied the excretion of radio-active iron after intravenous injection in the dog, observed three to fifteen days after injection a total urine excretion of 2–8% of the amount injected. After that there was no more excretion through the kidneys, whereas throughout the entire period of the investigation the stools contained iron. If the erythrocytes are destroyed by phenylhydrazine, the iron content of the stools increases, most of the excretion being effected through the biliary system; but where there is no haemolysis this is minimal (0.1 mg. daily, or even less).

It is thus seen that iron excretion is but slight, both in normal and anaesthetised dogs. In human beings we observe increased iron output through the bile in the course of haemolysis. In the

case of one of our patients the iron content of the bile rose from 100 to 250 γ% under the influence of phenylhydrazine treatment, dropping to the initial content at the end of the treatment.

As a general rule it can be concluded from the uniform results obtained from a series of detailed and laborious experiments (*Reinmann, F., Fritsch, F.* and *Schick, K.* in 1936; *McCance* and *Widdowson* in 1938; *G. H. Whipple* in 1939) that iron excretion is a function of secondary interest lacking major physiological significance, in view of the fact that the organism maintains its iron balance principally through the control of absorption.

(*d*) *New Research in Iron Metabolism Carried out with the Help of Radio-active Iron*

Since radio-active iron possesses the same chemical characteristics as iron itself, it has been used in recent years as an element of preference in the study of metabolism, owing to its physical property of radio-activity, and it has served to solve several problems associated with the action of this metal within the organism.

Certain American authors, particularly *Hahn, Whipple* and collaborators, *Moore* and collaborators, and *Ross* and *Chapin*, were able to demonstrate that if radio-active iron is administered *per os* the intestinal absorption of the metal is insignificant, but that it is increased if the organism needs additional supplies of iron, as for instance in the various forms of anaemia.

If the absorption of iron through the intestine is slight, the excretion of iron is also particularly low; actually it can be observed that traces of radio-active iron leave the organism, either directly or by excretion through the intestinal mucous membrane, or through the bile, which always contains very small quantities of iron.

Furthermore *Hawkins* and *Hahn* showed that the excretion of iron in the bile is definitely augmented during experimental haemolysis, but remains low compared with the marked increase of bilirubin excretion. Hence it must be assumed that a great proportion of the iron liberated by the destruction of haemoglobin is retained in the liver. The liver thus exercises a regulatory function in the excretion of this metal, although this excretion is still dependent upon intestinal absorption. As a matter of fact, as will be seen in pages 166–188, the iron may move in a double entero-hepatic circuit: excreted by the bile, it may be partially absorbed again through the intestine and reach the liver through the portal vein. This double circuit doubtless possesses a biological value. It shows, above all, the effort made by the organism to avoid the excretion of its iron; and this phenomenon

can doubtless also be interpreted as a mechanism for the transformation of iron which has been excreted and which, biologically inactive, reassumes through intestinal absorption its properties of an iron rendered active for the organism.

But the problem which must be of special interest for the physiologist and the clinician is that relating to the subsequent transformations of the iron, once it has passed the intestinal filter. It is to this problem that we have given most attention, especially in recent times, by the utilisation of radio-active iron, not given *per os*, but by direct intravenous injection into the circulation, either in a bivalent or a trivalent form, that is, in a reduced stabilised form or an oxidised form. These experiments were conducted on rabbits.

A few hours after injection nearly all the iron is restored to the organs.

The blood plasma is particularly rich in this iron; the red cells have a greater amount than at first, but after three days the value of the plasma diminishes and the erythrocytes become richer in iron. (*Miller* and *Hahn* found that radio-active iron was rapidly incorporated in the red cells.)

If the doses of iron are considerable (2–3 mg.) we observe a great and rapid excretion through the kidneys, manifested as soon as one and a half hours and even half an hour after the injection. The kidney possesses the power of rapidly excreting the iron as soon as the content of serum iron attains a certain level. Nevertheless the limit of renal excretion is particularly high. As soon as the iron is fixed in the erythrocytes it can no longer be excreted through the kidneys. On the other hand, excretion through the bile occurs more slowly and in fairly small amounts.

During the first few hours following upon injection we observe a difference in the behaviour of the bivalent and the trivalent iron.

The trivalent iron is rapidly excreted in the urine in much greater amounts during the first few hours after injection, whereas the bivalent iron is excreted in smaller quantities and more slowly. The duration of the trivalent iron excretion is usually shorter (1–2 days) than that of the bivalent iron, in which excretion proceeds at first in small amounts but lasts longer (2–3 days).

Associated with this difference of excretion we note a more rapid decline of the trivalent iron in the plasma, attaining its maximal values three hours after injection. The passage of the iron from the plasma to the red cells is fairly rapid, but three days after injection a decided decrease of the trivalent iron can be noted in the plasma and the erythrocytes.

The bivalent iron, on the other hand, shows a slow diminution of its content in the plasma, corresponding to a progressive

enrichment of the red cells in iron. This fact emphasises the difference in the intermediary metabolism of the bivalent and trivalent iron—a difference which must be clinically recognised in order to determine what form of iron should be injected.

The organ which participates most actively in iron metabolism is the liver, which becomes rapidly enriched after the injection of trivalent iron. While this occurs less rapidly during the first hours it proceeds more quickly three days after the injection of bivalent iron. After three days the liver shows a diminution of its trivalent iron content, corresponding to more abundant elimination in the bile.

The spleen hardly participates at all in iron metabolism if the iron is injected intravenously. In healthy rabbits the muscular system does not appear to take any active part in iron metabolism immediately after injection.

After ten days the blood plasma contains practically no iron at all, but on the other hand, the red corpuscles are particularly well supplied. We were interested to discover whether this iron was bound up with the stroma of the erythrocytes or whether it already constituted part of the blood pigment.

We therefore extracted the haemoglobin from the erythrocytes and transformed it into crystalline haemin, as a result of which we were able to detect the presence of a radio-active haemoglobin, that is, the appearance of radio-active iron within the molecule of the blood pigment.

The appearance of iron in the blood pigment of normal animals thus occurs toward the end of the first week, when the liver has attained its maximal content of iron. From that time on, the metabolism of iron in animals which have received trivalent iron is comparable to that of animals which have received bivalent iron. Continuing the study of iron metabolism twenty and thirty days after injection, we determined that during the second week the content of hepatic iron diminished, whilst that of radio-active haemoglobin continued to increase, attaining its maximal values at the end of the fourth week. This fact was indicated by an enrichment of radio-active iron in the bone-marrow after thirty days, whilst the iron level remained low for ten to twenty days during the phase of maximal haemoglobin synthesis.

It is interesting to note that the passage of the iron from the liver to the bone-marrow between the tenth and twentieth days was characterised by a fresh rise of the radio-active iron content in the plasma—a value which had fallen to nearly zero after the first three days.

An interesting fact to be stressed is the distinct increase of iron in the bile towards the thirtieth day and the augmentation of

iron in the liver after one month, a condition probably corresponding to the start of haemolysis of the erythrocytes containing radio-active haemoglobin. Actually it was during this time that the spleen also increased its content of radio-active iron. Here we will mention the work of *Curz, Hahn* and *Bale*, who found that, after disintegration of the red cells, the liberated iron appeared almost to be quantitatively utilised for the regeneration of haemoglobin.

Finally, iron also participates in the formation of the cellular haemins, but while the synthesis of radio-active haemoglobin is accomplished after a week, myoglobin and cytochrome synthesis probably occurs more slowly. Thus we were only able to observe radio-active myoglobin thirty days after iron injection.

In anaemic rabbits iron metabolism is especially intense. The erythrocytes become greatly enriched in iron a few hours after injection, but the excretion through the urine is less than in normal animals. The bile contains only small quantities of radio-active iron. The liver and bone-marrow become enriched in a very striking manner, particularly during the first few hours, and maintain a fairly high level during the first few days. It was interesting to observe the state of the iron in the musculature and above all in the myocardium. During the first few hours after injection the muscle, especially the cardiac muscle, became enriched in bivalent iron, whilst trivalent iron increased after the second or third day.

The appearance of radio-active haemoglobin is seen in anaemic rabbits as soon as a few days (2–5 days) after the injection of radio-active iron.

A hepatic lesion (phosphorus poisoning) provokes a definite diminution of the radio-active iron content in the liver and bile, with a slight augmentation in the circulating blood.

Finally we carried out in a normal animal a transfusion of blood containing radio-active haemoglobin and were able to observe the destruction of the molecule of radio-active haemoglobin in the organism. The erythrocytes introduced in the transfusion are able to remain in circulation a long time before being destroyed by the organism which receives them. In haemolysis the liver and spleen, and above all the bone-marrow, become enriched with radio-active iron. The bile is especially rich in iron —a fact which would indicate an increase of the destructive function of the haemoglobin at the level of the liver.

These observations have clearly shown us the important role played by the liver in the regulation of iron metabolism, and the secondary role of the spleen, which serves as an organ of storage only in the case of iron liberated during the destruction of the erythrocytes. Moreover, we can emphasise the importance of iron

metabolism in connection with the respiratory function of the tissues, as a result of the close collaboration between iron metabolism and the synthesis of the pigments possessing a haemin base. Henceforth we may not only be concerned with the metabolism of haemoglobin, the ferruginous pigment of the circulating blood, but also with the metabolism of the cellular haemins (oxydase, catalase, cytochromes, etc.), and we should study, as shown by our clinical and experimental investigations, not only the *circulatory anaemias*, but also the conditions of diminished cellular pigments, based on haemin, that is the *tissue anaemias*.

Finally, our investigations with radio-active iron have shown that the kidney may be an important organ for iron excretion if the iron is administered in large intravenous doses. In collaboration with *Carmine*, we studied this problem on human subjects and reached the conclusion that human beings can also excrete appreciable quantities of iron through the urine.

It seemed useful to us to compare the quantity of iron excreted in the urine with the content of serum iron one hour after injection. The increase of serum iron accompanying iron excretion varies between 180 and 300 γ%. The lowest level of serum iron we have been able to observe, accompanied by iron excretion (one single instance), was 150 γ%. Moreover, we were able to observe that the amount of iron excreted was proportional to the amount of serum iron.

The question thus arises as to whether the kidney is not actually an organ of iron excretion with the function of preventing too great an increase of iron in the blood and in the organism as a whole. Thus iron excretion through the kidney would be limited and proportional to the serum iron content.

In healthy individuals this limit is relatively constant, varying between serum iron levels of 200 and 250 γ%. In this connection we made an interesting observation on anaemic subjects and noted that the urinary excretion of iron in anaemic subjects was 20–30% less than in normal individuals and was accompanied by a definitely lower increase of serum iron than was observed in normal individuals. Hence it must be concluded that, strictly speaking, there does not exist a renal threshold of iron excretion, but that this established by the abrupt variation of the serum iron level, the level of serum iron at the time of iron injection being an additional determining factor.

Therefore, in anaemic subjects a sudden rise in the rate of the serum iron level from 10–20 γ% to 150 γ% suffices to provoke the appearance of iron in the urine, whilst in normal individuals an increase of 100 γ% to 200–250 γ% of the serum iron is necessary before the iron makes its appearance in the urine.

Thus the renal excretion of iron should be interpreted as a mechanism for the regulation of iron metabolism, the object of which is to avoid too abrupt and dangerous an increase of the level of the serum iron and of the iron in the organs.

(e) *The Effect of Age and Sex on Iron Metabolism.*

Recent work by *Widdowson* and *McCance* has indicated the importance of age and sex in iron metabolism. Obviously, the amount of iron contained in the body is not the same at all ages, neither is it equal in distribution and form. According to these authors the quantity of iron in the foetus rapidly increases during the last weeks of pregnancy. The figures indicate why it is that a child prematurely born so frequently suffers from anaemia.

Bunge was the first to determine that a newly-born animal possesses considerable iron reserves in its liver. But this only applies up to a certain point. *Widdowson* and *McCance* were actually able to show that the quantities of reserve iron found in the livers of a number of newly-born animals amounted to only 7–25% of the total body iron. This may appear a considerable amount, but actually it represents only a small fraction of the iron needed by the rapidly growing animal. The above-mentioned authors indicate the quantities and distribution of iron in the bodies of young pigs. First it is seen that at birth most of the iron existed in the form of haemoglobin. Actually the circulating haemoglobin contained 90% of the body iron, which, combined with the inorganic iron of the liver, represented nearly the whole of the body iron. 100 cc. of blood contained 28.3 mg. of iron. Three weeks later the entire picture had changed. The young pigs had quadrupled their weight and the total iron in their bodies had risen from 47.2 mg. to 125 mg. The percentage of iron in the liver had fallen considerably, but the total amount of iron mobilised from the liver for the needs of the body amounted to less than 3 mg. In the opinion of *Widdowson* and *McCance* this appeared to be an insignificant contribution to the iron needed by the animal. The circulating haemoglobin had increased during the first three weeks, and now amounted to approximately 60% of the total body iron. It was presumed that the remainder must be present in the tissues in the form of myoglobin or catalytic iron. During these three weeks the young pigs became very anaemic, 100 cc. of blood contained only 14.1 mg. of iron. In the eighth week the pigs finally got over the anaemia. The figures in the tables of *Widdowson* and *McCance* show that the circulating haemoglobin at that time contained only 384 mg. of iron, representing 78% of the total body iron. The iron in the liver rose from 1.8 to 23 mg. and in the rest of the body from 41 to 87 mg.

In 1936 *Steenbock, Semb* and *van Donk* offered a short communication regarding sexual differentiation in connection with iron storage in rats. They found that when young rats attained sexual maturity the females possessed greater supplies of iron than the males. During pregnancy these supplies were rapidly exhausted as the quantities of iron in the foetus increased. *Widdowson* and *McCance* were able to confirm and extend these observations of the American workers. They found that after birth there was a further rise in the concentration of iron in the livers of rats of both sexes, but that after the fiftieth day the livers of the females always contained more iron than did those of the males. Adult male rats are always larger than females of the same age, but if the sex glands are removed while they are young, both male and female rats grow at the same rate and, according to these authors, their livers contain equal quantities of iron. They found that the administration of Stilboestrol to female rats led to a slight reduction in the rate of growth and to an increased accumulation of iron in the liver. The administration of Stilboestrol to male rats and of Testosterone to the females gave rather vague results, indicating that the male and female hormones probably co-operate with certain factors which are not present in the body of the other sex.

In conclusion, we may add here that, according to numerous authors who have taken up this question (*Heilmeyer, Hemmeler* and others) it may be said that during the first year of life the quantity of iron necessary for the rapid growth of the organism is very great and must be about 1 mg. per day. In the subsequent years, on the other hand, the annual needs of iron should not exceed 100–400 mg. per year.

It is after puberty that the iron needs begin to vary in men and women. During menstruation women lose 20–50 mg. of iron, that is, about 300 mg. per year. During pregnancy and delivery, as well as during lactation, women lose about 500 mg. of iron. Pregnant women usually absorb two to ten times more iron than do non-pregnant women (*Balfour, Hahn, Bale, Pommerenke* and *Whipple*). Suddenly, after the menopause, the organism of women needs the same amounts of iron as that of men.

III. The Biological Significance of Iron

Iron, which is present in various forms in all tissues, is indispensable for cell formation and hence is intimately connected with body growth. However, iron needed for the normal course of cell oxidation also exercises a biocatalytic function which is of vital importance in cell respiration. Finally, as part of the haemoglobin molecule, this mineral plays a role of far-reaching importance in connection with erythropoiesis.

We shall therefore proceed to an examination of the three main biological functions of iron, and shall discuss separately its significance as a factor affecting (*a*) growth, (*b*) catalysis, and (*c*) erythropoiesis.

(*a*) *Iron as a Growth Factor*

Elvehjiem was able to demonstrate the part played by iron in the growth of brewer's yeast cultures. On a nutrient medium devoid of iron the culture does not develop well; the respiratory exchanges are inadequate, the cells are deficient in cytochrome. In such cases it suffices to add iron in order to observe a rapid resumption of growth. *Kauffmann-Cosla* and *Brüll* also point out the favourable influence that iron exerts on the growth of cultures of Aspergillus, a phenomenon due, in the opinion of *Sauton*, to the fact that iron favours spore formation in the cultures. According to *Lasseur* and *Thiry* iron promotes pigment formation in certain bacteria, as for instance, the tubercle bacillus.

The numerous clinical and experimental observations of iron metabolism in the newly-born clearly indicate the influence that this mineral has on growth. As early as at the end of last century *Bunge* drew attention to the fact that the newly-born start life with a considerable iron reserve; in their case the iron content of the liver is four to nine times greater than in adults. On the other hand, in the newly-born the spleen appears to contain very little iron (*Lapicque*). In their systematic studies of iron metabolism *Fontès* and *Thivolle* showed that in the rabbit the iron content remains constant during the first weeks of life; in the dog, on the other hand, the iron content of the liver rapidly increases 100% in a week during the period of lactation, an indication that the dog receives its rich supply of iron through its mother's milk. Thus the amount of iron reserves of the newly-born depends entirely upon the species of animal. The cat and rabbit, unlike the dog, possess great reserves of iron. Finally, it would appear that the iron reserves of the liver are only mobilised if the nutritive intake of iron is insufficient.

The iron reserves at the time of birth, as well as that which is to be taken in with the mother's milk, are of great significance

in connection with the growth of the organism. The quantities of iron which the mother gives up to the foetus during the last months of pregnancy are very considerable (*Hugounen*). *Hofbauer* even reaches the very interesting conclusion that the destruction of the red blood corpuscles in the placenta of the mother represents a very important means whereby the foetus is supplied with the quantity of iron needed for its growth. *Kottmann*, on the other hand, is convinced that the serum of the mother possesses the capacity of ionising the iron which it liberates from the organic complexes, and by this means of supplying the embryo with active iron.

Vahlquist determined, on the basis of very many observations, that the foetus possesses a quantity of serum iron of 21–70 $\gamma\%$ up to the sixth month, of 97–100 $\gamma\%$ in the eighth month, and of 146–161 $\gamma\%$ after the ninth month. These last values are higher than those of the mother, and this must be considered to correpond to the increased need of iron on the part of the foetus, particularly during the last months of its development.

These observations are all consistent; they accentuate the significance of iron as a substance of cell composition and growth promotion in the embryo. According to *Anselmino and Hoffmann* the increase of haemoglobin in the foetus corresponds to a necessary condition for oxygen saturation of the foetal blood. In the opinion of these authors the embryo lives under the same conditions as an organism in high altitudes, where the increased haemoglobin represents the physiological response to the lack of oxygen. Hence at the outset of extra-uterine life the newly-born would possess a surplus of haemoglobin. The sudden change which birth effects in the conditions of respiration would result in the immediate destruction of the excessive supplies of haemoglobin. This would explain Icterus neonatorum. But in our opinion this Icterus possesses an additional point of interest; for it would not only be necessary for the destruction of the excessive haemoglobin which has become superfluous, but it would at the same time provide the organism with large quantities of iron needed for its growth and for the enrichment of its reserves.

(*b*) *Iron as a Biocatalyst*

Our knowledge regarding cell respiration, long confined to the simple concept of ordinary combustion in the presence of oxygen, has undergone a fundamental change, especially in recent times. A number of highly important works appearing in close succession have demonstrated with steadily increasing scientific exactitude that the combustion of the nutrient substances, which must be interpreted as a source of energy in the organism, is

bound up with a series of chemical processes which for their part are liberated by the co-operation of various catalysts. The phenomenon of cell oxidation is not a continuous process, but is rather subject to the reactions of the environment and associated with the presence of substances which either facilitate or impede respiration.

An effort has been made by means of various theories and innumerable hypotheses to explain the complicated mechanism of cell oxidation (or utilisation of the oxygen circulating in the blood) in connection with cell respiration.

Suffice it here to mention only two theories which have long confronted each other: *Warburg's* theory relative to the activation of oxygen, and *Wieland's* theory which assumes that the chief mechanism of respiration is based on hydrogen removal from the substrate, i.e. dehydrogenation. To-day we know that both these processes are indispensable for cell respiration.

As a matter of fact, the oxygen can only be associated with the cellular substrate when the oxygen is activated (*Warburg*). This activation is effected by means of a catalyst: iron. Molecular oxygen actually reacts with divalent iron with the formation of trivalent oxidised iron which is capable of transferring its oxygen to the substrate. As soon as the substrate has been oxidised the trivalent iron is again reduced to divalent iron. The ability of iron to become rapidly oxidised or reduced as a result of a change of valency explains its great biological significance as a catalyst in cell oxidation.

According to *Warburg*, cell respiration is catalysis at the surface of the cell effected by iron. Hydrocyanic acid, which very easily forms stable complexes with the iron, is thus able to prevent the catalytic action of this metal and to impede certain processes of cell respiration.

On the other hand, the loss of hydrogen to the substrate can only take place if the hydrogen is activated by enzyme action. This activation is effected by dehydrogenases, and the liberated hydrogen is then conducted by special hydrogen-carriers to its final recipient. It is interesting to note that the group of hydrogen-carriers includes substances of the greatest biological and clinical interest.

Thus we find the catalyst system of *Szent-Györgyi*, Glutathion (*Hopkins*), Adrenalin, probably Ascorbic acid (Vitamin C), and above all the respiratory yellow enzyme in which Lactoflavin (Vitamin B_2) plays the part of co-ferment; finally there are the nicotinic acid enzymes (Vitamin PP).

The oxygen activation of *Warburg*, on the one hand, and *Wieland's* dehydrogenation of the substrate, on the other, would

not complete the cycle of the regulation of cell respiration. Between these two systems a third must be inserted, a system which has been examined and described by *Keilin* and which consists of cytochromes. The cytochromes are important tissue pigments which occupy an intermediary position between the processes of activation of oxygen and of hydrogen, respectively. They represent a complex combination of iron-porphyrin and are hence to be considered as tissue haemins. These respiratory pigments are found in comparatively large quantities in all cells which consume oxygen rapidly, and their amount is proportional to the intensity of respiration of the cell. That is why the muscle cell possesses a conspicuously high concentration of cytochromes.

The cytochrome is not auto-oxidisable, but its oxidation is activated by *Warburg's* respiratory red enzyme, cytochromoxidase. This enzyme, which is oxidised by oxygen activation, oxidises the cytochrome which is finally reduced at the level of the hydrogen-carrier. For that reason the cytochrome functions by virtue of the catalytic activity of its iron as an electron-carrier and completes the chain which unites the two systems of *Warburg* and *Wieland*.

We can therefore illustrate the regulation of the processes of respiration as follows:

Cell substrate — Dehydrogenases $\xrightarrow{H_2}$	Hydrogen carriers	$\longrightarrow$ Cytochrome —	Cytochrome-Oxidase $\longleftarrow$ O
Hexoses, Trioses, Aminoacids, etc. H_2	Diaphorase, *Szent-Györgyi*-system, Glutathione, Adrenalin, Ascorbic acid, Nicotinic acid co-enzymes Warburg's respiratory red enzyme		

As a hydrogen-acceptor oxygen is able to form hydrogen-peroxide in the tissues.

As H_2O_2 is dangerous for cell life the organism is compelled to destroy this substance quickly. This is effected by the catalase which is present in the cell—an enzyme which, like cytochrome and oxidase, belongs to a group of tissue haemins and whose catalytic activity in its turn is due to its iron content.

Thus we see that there exist in the cell a number of iron-

containing pigments, all of which manifest the chemical character of haemin, and consequently, from a chemical point of view, show certain closer relations with blood haemin (haemoglobin).

This fact compels us to assume that, in addition to a disturbance of haemoglobin-iron metabolism, there must exist cell-haemin metabolism. This matter will subsequently be dealt with at length.

It should be mentioned that although the haemoglobin-iron contained in the blood develops no catalytic activity, the possibility is by no means excluded that under certain conditions an exchange of gases may take place in the circulating blood. In the opinion of *Starkenstein* that would occur in the oxidation of divalent into trivalent iron in the blood stream. This iron is associated with the globulins, whereby the iron-globulin complex forms an iron-protective system. This iron complex is conveyed by the blood to the tissues where the trivalent iron of the globulin complex is liberated (*Starkenstein*) and can be utilised as a catalyst either directly or through the mediation of the respiratory ferments.

Lewis observed in yeast (Torulopsis utilis) the fact (of importance in connection with the functional relationship between iron and vitamins of the B group) that lack of iron resulted in increased synthesis of thiamine, riboflavine, nicotinic acid, and pyridoxine, but decreased the rate of synthesis of biotine and para-aminobenzoic acid.

(c) *Iron as a Factor in Blood Formation*

Our knowledge regarding erythropoiesis is still defective. The renewal of blood is based upon equilibrium between the formation of new erythrocytes and the destruction of the old ones by haemolysis. The mechanism of this physiological destruction is still unclarified; it is based in part upon the wearing out of the blood corpuscles in circulation, mainly as a result of mechanical factors; but in part it is due to certain enzymatic processes of destruction. The reticulo-endothelial system, particularly the spleen, is clearly connected with this blood decomposition.

The mechanism of erythrocyte formation, or erythropoiesis, is hardly less complicated and vague. The red blood corpuscles are formed in the bone-marrow, where haemoglobin is only gradually produced as the erythrocytes proceed to maturity. Actually the cells of the red group do not all hold the iron-containing pigment. The following table of *Dreyfus* clearly indicates a development of the iron-containing blood pigment from the polychromatophile erythroblast:

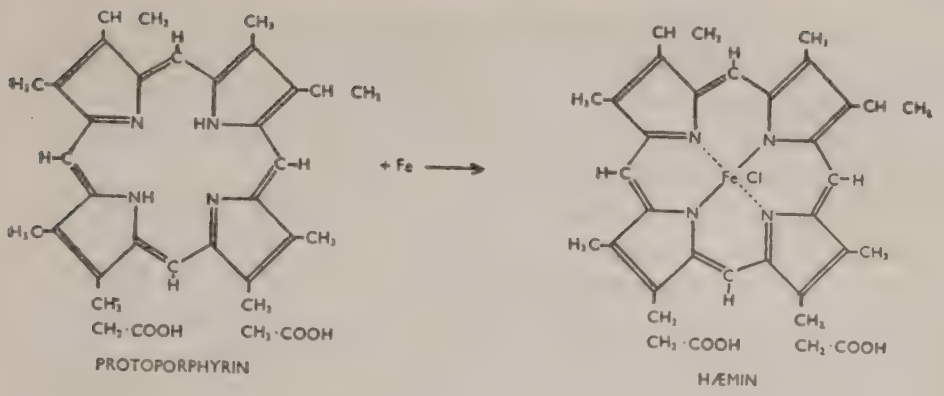
Haemocytoblast (original cell) Proerythroblast Basophile erythroblast	without iron-containing pigment
Polychromatophile erythroblast Normoblast Reticulocyte Erythrocyte	with iron-containing pigment

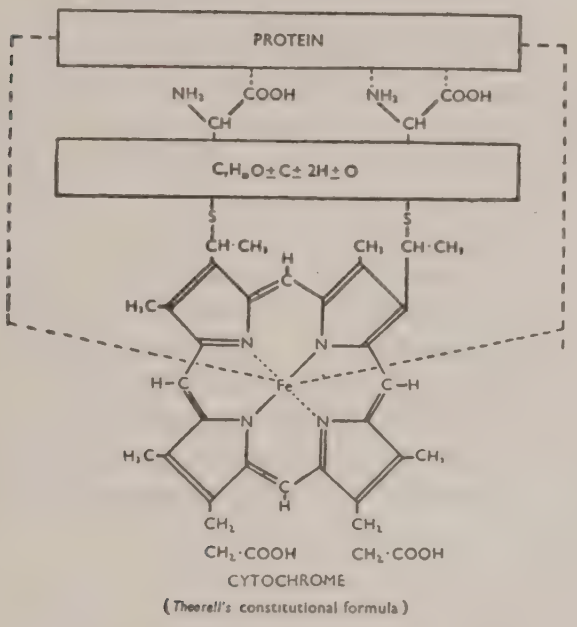
The progressive concentration of haemoglobin in the erythroblast gives a certain indication of the stage of maturity of the cell; it is this also that starts the karyolysis. In all probability the haemoglobin is produced under control of the erythroblast, which already possesses iron in its cytoplasm. Thus the nucleus, according to *Macallum*, is the agent which introduces the iron into the chromogen that as yet has none. This catalytic activity of the nucleus would explain the karyolysis which follows next in haemoglobin formation, as well as the fact that after karyolysis the red cell loses the power of forming haemoglobin.

The iron introduced into the organism in nutrition can therefore serve for the purpose of haemoglobin formation only after it has undergone successive transformations. We must always be mindful of this fact, already stressed by *Starkenstein*, in our more detailed consideration of the problem of iron metabolism in connection with haemoglobin formation. *Starkenstein* correctly envisages a possible analogy between the iron of the erythroblasts and that of the nucleus of the other tissues. In the bone-marrow this iron would lead to haemoglobin formation, in the other tissues to the formation of other cellular haemins, which are indispensable for respiration and for the manifold biochemical processes of cellular metabolism.

With the help of radioactive iron and centrifuging to separate the cytoplasm from the nucleus after *Claude's method*, we were able to observe that more iron is found in the cytoplasm. In lead poisoning, which is accompanied by a great production of protoporphyrin, this pigment is also found in great quantity in the cytoplasm and in small quantities in nucleus. Thus, we must admit that the synthesis of the haemoglobin from iron and protoporphyrin is accomplished in the cytoplasm of the erthyroblast. This is also shown by the histological staining of the haemoglobin in the erythroblast of the bone-marrow. It is possible that copper may be associated, as catalyst, for this mechanism.

During the first months of embryonic life the organism is not capable of providing for complete haemoglobin synthesis; this remains at the stage of chromogen without iron, i.e., of a porphyrin, and only at a later stage is the iron used for the





building up of the normal blood pigment. As has been proved by *Borst and Königsdörfer*, in human pathology there exists a form of erythropoiesis which, phylogenetically and ontogenetically considered, represents a retrogression to the embryonic stage of haemoglobin formation. This is pernicious anaemia, in which the partial lack of karyolysis is distinguished by the appearance of porphyrin and by an enrichment in iron of the circulating blood and of the tissues.

Finally we wish to recall the hypothesis of *Amano* (see page 160) who, proceeding from the determination of a high content of cytochrome in the eosinophile leucocytes, assumes that these cells serve primarily for the transportation and the deposit of this iron-containing pigment, so essential for the mechanism of respiration. In this way one is led to consider the biological activity of iron as follows: *This mineral represents a normal and indispensable*

constituent of the cell nucleus, destined to unite with the corresponding haemochromogen, by forming, on the one hand, haemoglobin, and on the other, cellular haemin, i.e. cytochrome, and probably also other haemin-containing substances of cellular metabolism and cell respiration.

Without going into the subject, we would like to point out briefly here the modern conceptions on the formation of porphyrins during the synthesis of the haemoglobin (see *Dobriner* and *Rhoads, Watson, Rimington,* etc.). According to these authors, porphyrin is formed by the joining of two groups of pyrromethene. This may produce different combinations, either characteristic of porphyrin III, which, when it is joined to iron, produces haemoglobin, or of porphyrin I which, on the contrary, cannot be joined to iron. This latter porphyrin is always found in very small quantities in the normal organism and may increase in pathological states (pernicious anaemia, porphyria). The quantity of porphyrin III often increases in cases of considerable blood regeneration, with the lack of iron, or in cases where iron cannot enter into the porphyrin ring (see further).

It seems that copper plays an important part in the introduction of iron into the porphyrin ring. *Copp* and *Greenberg* observed actually that the utilisation of iron in the formation of haemoglobin is increased by the presence of copper. Finally, it is interesting to note that with the help of glycine marked by radioactive N, *London, Shemin* and *Rittenberg* showed that this amino-acid begins to form part of the protoporphyrin and remains there till the haemoglobin is destroyed.

IV. The Circulating Iron

In addition to the iron found in the tissues as reserve, the iron serving as biological catalyst, and the iron representing a constitutional element of the cell, there remains for our consideration that fraction of iron which is present in the circulating mass of blood. This iron is for the greater part bound up with the molecule of haemoglobin. It is the haemoglobin iron. But in addition to this iron fraction the blood contains another fraction which does not belong to the molecule of the blood pigment and which is therefore designated *non-haemoglobin iron.* Thus the blood whole plays the part of an iron-carrier, for which reason this fraction of iron circulating in the blood which is unconnected with haemoglobin has been called *transport-iron.*

The discovery of a non-haemoglobin iron fraction is relatively recent. For a long time it was overlooked in making quantitative determinations of iron, because in comparison with haemoglobin iron its amount was very small. As late as 1891 *Socin* found no iron in the blood serum, and not until 1898 did *Abderhalden* discover that the total iron of the blood exceeded the values calculated from the haemoglobin content, from which he inferred that the blood must contain some iron unconnected with haemoglobin. One year later *Häsermann* discovered iron in non-haemolysed blood plasma. Subsequently *Fowell* confirmed *Abderhalden's* observations and determined by means of quantitative analyses the relations between the haemoglobin iron and the transport iron in the blood. In normal individuals this relation is 4.2 : 1, in secondary anaemia 3.7 : 1, and in pernicious anaemia 2 : 1. In France *Fontès and Thivolle* conducted very painstaking examinations to determine the various fractions of iron present in the blood. They, too, were able to confirm the permanent presence of a non-haemoglobin iron fraction in the serum. In secondary anaemia, induced in the horse by bleeding, this serum iron fraction was found to be definitely reduced. *Henriques and Roche* designated as " non-haemoglobin iron " that iron fraction which is independent of the blood pigment iron. These authors calculated that in the horse the amount of non-haemoglobin iron in the serum is approximately 1–2 mg. per litre; the same amount was found in the pig, calf, dog, cat and rabbit. In the opinion of *Jenkins and Thomson* the non-haemoglobin iron represents approximately 7% of the haemoglobin iron present in the erythrocytes.

Barkan has the merit of having studied in a systematic and extremely thorough manner the non-haemoglobin iron fraction in the circulating blood. This author observed that, if hydrochloric acid was allowed to act on blood, small quantities of ionised iron

were rapidly transferred to the filtrate. If the mixture of hydrochloric acid and blood was kept for twenty-four hours at a temperature of 37° the filtrate contained approximately 1.7 mg.% of iron, that is, a quantity corresponding to 5–6% of the previous haemoglobin iron. If pepsin or pancreatin was allowed to act on the blood, without the addition of hydrochloric acid, this phenomenon did not occur. This iron is present in the stroma of the red blood corpuscles, independent of the haemoglobin; for the red cells, after being washed and completely freed of their haemoglobin, still contain iron. Therefore he held that this iron fraction is not associated with the presence of haemoglobin; it is found again in the blood plasma and even shows a certain increase if the plasma is left for some time out of circulation. This increase is probably due to a process of migration of the iron of the red cells into the plasma. Moreover, the concentration of this fraction is quite independent of the haemoglobin content of the blood. *Barkan* calculated that in 1.0 g. of dried red blood corpuscles there is approximately 0.15 mg. of iron which does not belong to the haemoglobin. This observation is doubly interesting from the fact that, according to *Warburg's* determinations, about the same values of iron can be found in the various tissues and organs. This iron might thus be compared, quantitatively at least, to the respiration iron of the red blood cells. Nevertheless the increased respiration of the red corpuscles in the course of anaemia or of blood regeneration does not depend upon this fraction of iron. Another view ascribes this iron fraction to the transport iron which passes from one tissue to another, as for instance, from an organ of storage to an organ of consumption. Functionally considered, the non-haemoglobin iron of the red corpuscles would be identical in nature with the transport iron of the serum.

The observations of *Barkan* have called forth countless criticisms. Thus *Lintzel*, and also *Legge* and *Lemberg*, are of the opinion that the iron fraction isolated by *Barkan* and designated by him as "iron that can be easily split off" is an artificial product derived from the action of hydrochloric acid on haemoglobin. Hence *Barkan's* "iron that can be easily split off" would be of no practical significance. It would be a product of haemoglobin decomposition in which the hydrochloric acid converted the oxidised haemoglobin into methaemoglobin. Barkan's reply to this was that there exists no quantitative relationship between his iron fraction in the blood and the concentration of the haemoglobin. Moreover, that if dilute HCl is allowed to act on crystallised haemoglobin or on a solution of haemoglobin (prepared from haemoglobin crystals) the Barkan

fraction is not found. Other authors maintained that Barkan's iron is an artificial product, since from the time that the HCl is placed in contact with the blood the quantity of non-haemoglobin iron increases.

However, we can admit to-day that in the plasma there is a fraction of iron which does not belong to the haemoglobin molecule and which circulates with the blood proteins. We must definitely assume that the iron is partially adsorbed by the proteins of the plasma, but in part is actually chemically bound; certain fractions of it may also be free. This question may be of great significance since, as we shall see, the extraction and consequently the quantitive determination of iron may be subject to definite variations, dependent upon the physico-chemical condition of the iron in the plasma.

Until recently we possessed insufficiently clear indications regarding the combination of transport iron with the iron carriers. It is the supreme merit of *Cohn* to have demonstrated by means of his fractional technique that the iron carrier is chiefly β-globulin. At all events it is interesting to note that we are always concerned with at least two main forms of combination of iron with protein: adsorption and chemical binding. *Neukomm* has recently attempted to throw more light on this problem; he determined the iron bound to the protein as a function of the iso-electric point of the corresponding protein and concluded that the amount of iron taken up by the albumin depends on its iso-electric point. Moreover, the further the iso-electric point is shifted to the alkaline side, the less is the quantity of iron which is attached to the protein. As a result of the shifting of the iso-electric point, the chemical bonds are first loosened. According to *Neukomm*, albumen takes up more iron than does globulin. These investigations represent an attempt to elucidate the state of the iron with respect to its protein carriers (see *Laurell's* paper).

This fact has a great clinical importance, because it is not excluded that the percentage of the circulating iron depends partly on the amount and the composition of the plasma proteins, the same as their iso-electic point which is clinically related to the sedimentation rate of the erythrocytes.

Starkenstein and *Weden* have the merit of having separated the iron in the blood which is connected with haemoglobin from the inorganic iron, and of having thus determined that the circulating blood contains, not only haemoglobin iron, but also inorganic iron in the form of ferrous and ferric salts, in approximately equal parts. With the help of their method for fractional determination of the organic and inorganic iron these authors reached the conclusion that their inorganic fraction corresponds to *Barkan's*

" iron that can easily be split off " and also to the " non-haemoglobin iron " of other authors.

It is therefore obvious that, in addition to the iron which is bound up with the pyrrole nuclei and to that which forms haemoglobin, there exists yet another fraction of iron which is not united with the haemoglobin and does not belong to it. This then is iron which circulates in the whole blood, together with the iron belonging to the colouring matter of the blood and which is transported from one tissue or organ to another. This iron is variously designated, according to the different authors and their principles of classification. Thus *Henriques* and *Roche* speak of " non-haemoglobin iron "; *Warburg* and *Krebs* of " loosely bound iron ", since they observed in their determinations of tissue iron that this iron, contrary to what prevails with haemoglobin iron, is not closely bound up with the substrate. Utilising the same criterion of differentiation and in consequence of the facility with which it is extracted with the help of dilute HCl, *Barkan* speaks of an " iron that is easily split off "; *Starkenstein* and *Weden* use the expression " acid-soluble iron " or " inorganic iron ", in order to differentiate it from organic iron united to haemoglobin. *Guthmann, Brückner, Ehrenstein* and *Wagner* speak of an " ultrafilterable iron " of the serum. Finally, *Heilmeyer* in a series of recent clinical investigations has studied the content of iron in the serum liberated with the help of HCl and calls it simply " serum iron ".

It is obvious that all these various forms of designation relate to an iron fraction which is present in the whole blood, in the erythrocytes, in the plasma and in the serum, but which has no direct connection with haemoglobin. Nevertheless these manifold names do not convey an identical concept. They refer to fractions of iron obtained by various methods, and as we shall see below they frequently represent two different entities, considered from a chemico-analytical and biological point of view. All these fractions have one definite characteristic in common: they represent iron which is not connected with the haemoglobin molecule. For the purpose of characterising this group of fractions we shall employ the expression " non-haemoglobin iron ". Viewed from an analytical standpoint it might be well to stress the fact that this fraction can be isolated with the help of HCl and hence to speak of " acid-soluble iron ". We assume that the iron fraction in which we are interested is that which is being transported to the cell which needs it, which is on the way to storage depots, or, finally, to the organs of excretion. From a biological standpoint therefore the term " transport iron " or " circulating iron " would characterise this iron.

At this point we shall not dwell on the qualitative differences of this iron fraction, but will consider it in detail in a separate section. Here, however, it should be emphasised that there exists a certain uniformity regarding the values obtained by various means by different authors, a fact which argues again in favour of the existence of a fraction of blood iron which is neither artificially produced nor the product of the splitting up of the haemoglobin molecule by HCl.

The following table, taken from Starkenstein's monograph and completed by adding the results of recent findings, summarises the findings of a number of authors in connection with non-haemoglobin iron.

Authors	Animal	Substance	Non-haemoglobin iron mg. per litre
Barkan	ox	blood	17
Barkan	rabbit	blood	12–17
Starkenstein and Weden	rabbit	blood	19
Dominici	human being	blood	9–45
Häusermann	calf	plasma	10
Häusermann	ox	plasma	7–8
Barkan	rabbit	plasma	1.1–2.2
Barkan	dog	plasma	1.8
Starkenstein and Weden	rabbit	plasma	11
Starkenstein and Weden	human being	plasma	6.5
Barkan	rabbit	serum	2.24
Barkan	dog	serum	1.68
Fontès and Thivolle ..	horse	serum	1.92–2.08
Henriques and Roche ..	rabbit	serum	1.89–3.10
Henriques and Roche ..	cat	serum	1.62–3.01
Henriques and Roche ..	horse	serum	1.19–2.20
Henriques and Roche ..	pig	serum	1.92–2.40
Warburg and Krebs ..	dog	serum	2.12–2.67
Warburg and Krebs ..	rabbit	serum	1.13
Warburg and Krebs ..	human being	serum	0.67–1.16
Heilmeyer and Plötner	man	serum	1.26
Heilmeyer and Plötner	woman	serum	0.89
Locke, Main and Rosbach	man	serum	1.0
Locke, Main and Rosbach	woman	serum	0.89
Riecker and Winters ..	dog	serum	10
Langer	human being	serum	0.5–1.8
Guthmann, Brückner, Ehrenstein and Wagner	human being	serum	0.67
Moore, Arrowsmith ..	human being	serum and plasma	0.94–1.74
Quilligon and Read ..	woman	serum and plasma	0.58–1.42
Fowweather	man	serum	1.25
Fowweather	woman	serum	1.05
Van Goidsenhoven ..	man	serum	1.41
Hoet and Lederer ..	woman	serum	1.18
Vahlquist	man	serum	1.42
Vahlquist	woman	serum	1.23

Among the iron fractions which have been studied in greatest detail, the principal ones are *Barkan's* " easily split-off iron " and *Heilmeyer's* " serum iron ". We have mentioned above the significance of Barkan's researches and the recent works of *Heilmeyer,* which are of particular clinical value. In order that the investigations of these two authors may be better understood we shall at this point discuss separately and in detail both *Barkan's* and *Heilmeyer's* iron fractions.

(*a*) *Barkan's " easily split-off iron "*

As has been stated above, it is to the credit of *Barkan* to have shown the presence in the blood of an iron fraction, unconnected with haemoglobin, and to have devised a comparatively simple method of extraction and one that can be used in clinical analyses. *Barkan* endeavoured to indicate the nature and origin of this iron which he isolated. The circulating blood contains, in addition to haemoglobin, a number of iron-containing substances which differ from blood pigment and its products of decomposition. For instance, this applies to the prosthetic group of blood catalase, which is a prothaemin (*Stern*) and hence contains iron.

The " easily split-off iron " possesses characteristic chemical properties, which *Barkan* has pointed out. Two thirds of *Barkan* iron can bind oxygen and carbon dioxide, interchangeably, in the same way as is done by haemoglobin. *Barkan* indicates this fraction by E. The relationships which bind E to O_2 and CO_2 are characterised by the following equation:

$$\frac{O_2E}{CO_2E} = K_E \frac{O_2}{CO_2}.$$

We are therefore concerned with an equation which is similar to that constituting the bases of the relations between O_2 and CO_2 with haemoglobin:

$$\frac{O_2Hb}{CO_2Hb} = K_{Hb} \frac{O_2}{CO_2}.$$

These two equations differ only in the value of the constant K, which is different for E and for Hb. The affinity of E for CO_2 is four times greater than for haemoglobin. In the oxidised form alone, that is, in the form O_2E, the iron is easily separated from E.

Only a third of the Barkan iron is unable to bind oxygen or carbonic oxide. In order to distinguish this fraction from the preceding one *Barkan* designated it E_1. By adsorption with aluminium-oxide he separates the fraction E from the haemoglobin. Upon continuing his studies in connection with the properties

of E and E_1 he noticed that these two fractions reacted differently towards hydrochloric acid. As we saw above, this acid inhibits the catalytic action of iron during respiration, but this inhibition varies according to whether the iron is mono-, di- or trivalent. The hydrocyanic acid is much more active towards trivalent iron salts. Hence it exerts a stronger influence upon methaemoglobin (trivalent iron) than upon normal haemoglobin (divalent iron). *Barkan* was able to observe that the hydrocyanic acid acts principally upon E_1 and very slightly on E. Therefore he assumed that the iron present in E was divalent, that in E_1 trivalent.

While pursuing his studies in connection with the nature of the protein group which accompanies the iron, *Barkan* endeavoured to separate the iron E from the haemoglobin by means of kataphoresis. His inability to do so led the author to assume that the protein of E must be identical in nature with the globin of blood pigment. If the iron of the pyrrole nucleus is separated from the haemoglobin a porphyrin is produced. Haemoglobin is a porphyrin which contains an atom of iron within its molecule. But if one tries to split off the iron from E or E_1 no porphyrin is obtained. In the opinions of *Barkan* and *Lintzel* this observation serves to show that the prosthetic group of E does not possess a closed porphyrin nuclear structure, as does haemoglobin. They believe that E and E_1 possess a haemin structure which, however, is open, as is that of bilirubin, with the difference that the open ring of E and E_1 still contains iron.

Barkan names E and E_1 " pseudohaemoglobins ", by which name he understands substances which, like haemoglobin, consist of a protein body of the same nature as the globin of blood pigment, and of a prosthetic group corresponding to a haemin molecule, with the difference that it has an open pyrrole ring. It was therefore a pyrrole ring similar in type to that which *Lemberg* found in the green haemin of *Warburg* and *Negelein*. The splitting up of the haemin-porphyrin ring would make the union of the iron more loose. In this way it would be easier to comprehend the fact that the iron can be liberated by the simple action of dilute HCl, and this would explain the origin of the " easily split-off iron ". Nevertheless, it must be stated that the pseudo-haemoglobin contains an additional protein body, which is not the case with the green haemin, which is already a haemochromogen. These pseudohaemoglobins E and E_1 should therefore be considered as intermediary substances on the way to physiological transformation of haemoglobin into bilirubin.

Barkan is of the opinion that the first stage is a splitting off through oxidation of the haemoglobin-porphyrin ring in posi-

tion α. Through the oxidation of the pseudohaemoglobin E, the divalent iron is converted into trivalent (E_1) iron. Through reduction of the intermediary group CH into CH_2 and the liberation of the iron E_1, there would finally be effected the separation of the bilirubin, iron and globin. The iron and bilirubin would then pass from the red blood corpuscles to the blood plasma by the formation of the two following complexes: iron-globulin and bilirubin-albumin.

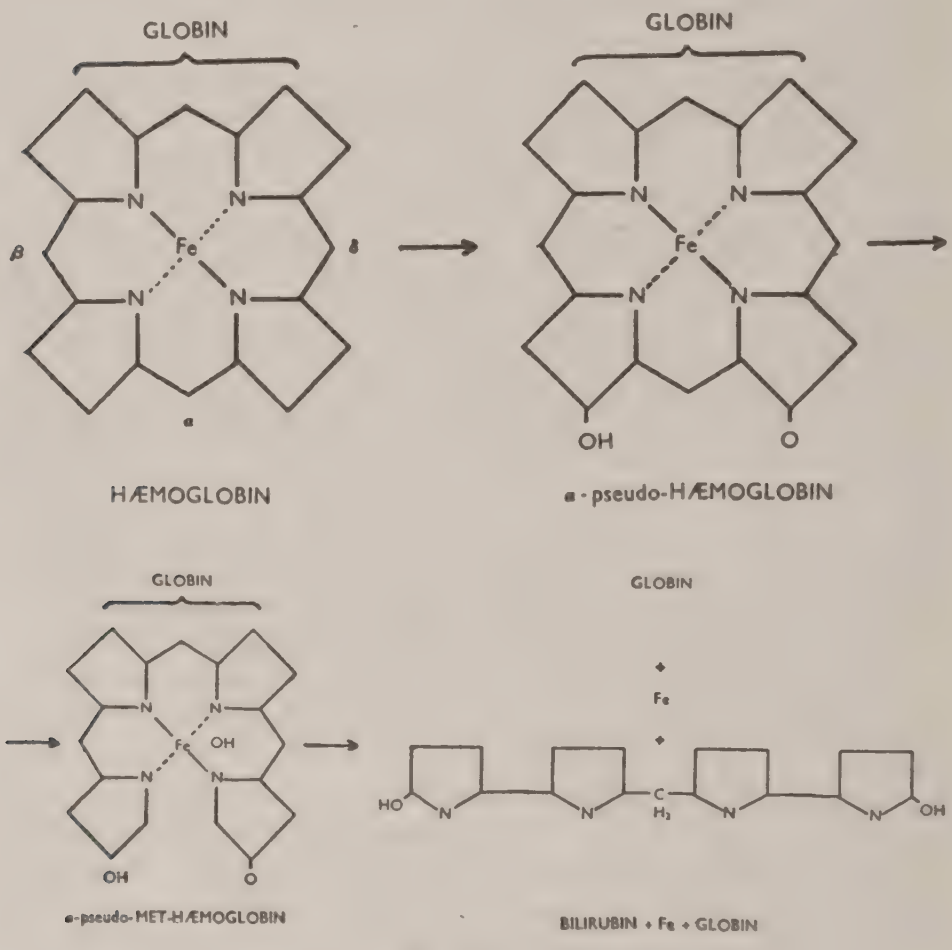


DIAGRAM 3
Formation of pseudohaemoglobin

Hence E and E_1 would be present in the blood as such and the iron and bilirubin of the plasma would represent their products of decomposition. It would therefore be probable that bilirubin formation is not necessarily connected with the activity of the reticulo-endothelium, but that it also takes place in the circulating blood. The research of *Aschoff* supports this view by demonstrating that the bilirubin is not formed exclusively in the reticulo-

endothelial system, but also in the circulating blood. The investigations of *Czike* also support this hypothesis and show that bilirubin formation can take place in the red blood cells. Thus the pseudohaemoglobin of *Barkan* would be identical with the verdohaemoglobin of *Lemberg* (*Engel*).

In addition to *Barkan's* iron fraction, a circulating iron fraction is found which is closely connected with the respiratory enzymes —iron which is important from a biocatalytic point of view. As a matter of fact the cell stroma is rich in active catalytic iron. *Warburg* showed this in his studies concerning the prominent role played by iron in oxygen transportation from the haemoglobin to the cell. Such iron is probably also found in the red blood corpuscles. *Ellinger* and *Landsberger* and later *Barkan* showed that fragments of red blood corpuscles, from which haemoglobin had been completely excluded, contained iron showing catalytic properties during cell respiration.

Finally it should be stated at this point that *Barkan* and *Schales* recently detected in the blood a haemin that is soluble in water and the spectrum of which is very similar to that of the prosthetic group of cytochrome C. Thus it is by no means impossible that this haemin C is a product of haemoglobin which, being soluble in water, is capable of penetrating through the cell membranes, to settle in the stroma and to form cytochrome C, in conjunction with a protein.

In addition to haemoglobin the blood contains a number of substances which are supplied with iron. *Barkan* groups them as follows:

1*st group*: Substances which as a prosthetic group possess a protohaemin and as a protein group possess a protein body differing from globin (e.g. catalase).

2*nd group*: Substances with a prosthetic group different from that of protohaemin, but with globin as protein group (factors E and E_1 of *Barkan*—pseudohaemoglobin, cytochrome C).

3*rd group*: Substances with a prosthetic group different from protohaemin and a protein group different from globin (*Warburg's* respiratory red enzyme).

For the sake of greater exactitude we will describe the original method of *Barkan* for the determination of iron in blood. plasma and serum. The iron is extracted with 1.2% HCl. The principle of determination is to form a thiocyanate-iron[1] complex characterised by a certain colour. The intensity of this coloration permits a quantitative reading to be made by comparison with freshly prepared solutions with a known iron content.

[1] Rhodanate-iron.

Technique: (*a*) *For Serum and Plasma*: Remove with a pipette 2 cc. of serum or plasma into a small centrifuge glass. Add 1.0 cc. of 1.2% HCl and allow it to stand for an hour at a temperature of 15–20°. Next, add 1.0 cc of 20% trichlor-acetic acid and stand for an hour at 15–20°. Centrifuge, decant (or filter with " Schwarzbandfilter No. 589 ", Schleicher and Schüll, of 5 cm. diameter).

(*b*) *For Blood*: The blood is defibrinated and haemolysed with a five-fold dilution with distilled water. To 1.5–2 cc. of the blood solution add half of the volume of 1.2% HCl, and place the mixture in the thermostat for 16 to 24 hours. Next, add 1.0 cc. of 20% trichlor-acetic acid and leave to stand for 1 hour. Centrifuge and decant, or filter.

The same procedure is adopted for blood, plasma or serum. 1 cc. of the decanted fluid or of the filtrate is placed in a test-tube fitted with a glass stopper. To this is added 1.0 cc. of a 10% ammonium-rhodanate solution and 1.0 cc. of peroxide-ether. The test-tubes are immediately sealed and inverted once or twice. The gases are allowed to escape by removing the stopper, after which the test-tube is quickly closed again. After standing for 10 minutes the closed glasses are inverted ten times. If the layer of ether is well separated from the remainder of the solutions the test-tube to be examined is placed in a comparator between the two test-tubes which it most resembles as regards intensity of colour. The solutions to be compared are prepared at the moment of the analysis with the help of a standard solution of trivalent iron with known Fe content. The iron solutions of known increasing concentration are placed in test-tubes provided with glass stoppers, then ammonium-rhodanate and peroxide-ether in the same proportions as in the solutions to be determined are added. Owing to the dilutions, the values obtained for the serum and plasma must be doubled and those obtained for the blood must be multiplied by 10. A more exact quantitative determination can be undertaken with a previously standardised photometer.

(*b*) *Heilmeyer's " Serum Iron "*

Heilmeyer extracts the serum iron with 6N HCl and does not permit contact of the serum and acid to exceed ten minutes. He then tests by the phenanthroline reaction, in which iron is characterised by a definite colour. The normal figures of the iron thus obtained are 126 γ% in men and 89 γ% in women. This difference between the two sexes is not the direct result of the physiologically lower content of the haemoglobin and red blood cells in women (actually the difference for these values is only 15%); but the 30% deficiency of serum iron seen in women is rather to be ascribed to the physiological loss of blood during menstruation. *Heilmeyer* is even led to believe that the deficiency of haemoglobin and red blood cells in women, as compared with that in men, is the consequence of repeated loss of blood through menstruation. A proof of this hypothesis would be found in the fact that the haemoglobin and iron values are about the same in both sexes in the periods before puberty and after the menopause.

The values of *Heilmeyer's* serum iron are constant; its content is not influenced either by food intake or by the time of blood removal. Repeated analyses made on the same individual during several days showed maximal oscillations of 30%. On the other

hand, the serum iron varies considerably according to the disease. A few days after a severe haemorrhage the iron content of the serum is very low, but this deficiency is not directly proportional to that of the haemoglobin. This considerable lowering is not necessarily the direct consequence of loss of iron from haemorrhage, but rather of reactive hyper-functioning of the bone-marrow. Nevertheless the iron content still depends greatly upon the degree and the nature of the haemorrhage (acute or chronic occurrence). In chronic haemorrhages the serum iron content is very low, very probably owing to the great lack of circulating iron. In haemolysis, on the contrary, the serum iron is considerably increased, whilst as a general rule in infectious diseases it is reduced. This decrease, in the opinion of *Heilmeyer*, corresponds to an increased need of iron on the part of the tissues for the work of combating the infection.

In the numerous investigations which he conducted *Heilmeyer* noted a decrease of serum iron in the following diseases: in acute and chronic haemorrhage; in achlorhydric anaemia; in pernicious anaemia during the regenerative phase following upon liver treatment; in acute infections; in tuberculosis, rheumatism, malignant tumours and lymphogranulomatosis. This author noted a reactive increase of the serum iron soon after acute haemorrhages, and the same thing was observed in the various forms of haemolytic anaemia and leukaemia, in haemochromatosis and in some cases of exophthalmic goitre.

A normal serum iron content is the expression of equilibrium between the sizes of the centripetal and centrifugal devices of circulation of the iron metabolism. The content of serum iron thus depends on the processes of absorption and excretion and upon the iron requirements of the reticulo-endothelial system and of the other organs and tissues. Finally, the bone-marrow and the entire haematopoietic system play an important part in the regulation of the serum iron.

The following is *Heilmeyer's* original method for the determination of serum iron:

2 cc. of serum is pipetted off in an Erlenmeyer flask. To this is added 1 cc. of 6N hydrochloric acid. It is well shaken and the precipitate is left to stand 10 minutes. Next, 2 cc. of 20% trichlor-acetic acid is added, the mixture is again shaken and allowed to stand for another 10 minutes; then filtered through an iron-free filter. Next 1 cc. of the filtrate, after the addition of 1 drop of 1% alcoholic solution of para-nitrophenol with 20% ammonia, is neutralised and acidified with N/2 H_2SO_4.[1] Then 1 drop of a 2% hydroquinone solution, with one drop of a 1% solution of ortho-phenanthroline-chloral hydrate is added and the development of the colour is awaited. The final volume can be easily measured. After 10 minutes the

[1] Of late, *Heilmeyer* has been using N/2 HCl.

absorption is read by the Pulfrich or Leifo photometer, with the filter 510 in the 5 cm.-high basins. The calculation of the photometric reading is made according to the following formula:

$(Ks.Vs - Kb.Vb) \times 1250 = \gamma$ iron in 100 cc. serum.

Ks = Extinction coefficient of the Phenanthroline-iron solution to be determined.

Vs = Final volume of the iron solution to be determined.

Kb = Extinction coefficient of the blank determination.

Vb = Final volume of the blank determination.

Besides *Heilmeyer's* method for determining serum iron, *Kühnau's* micro-method with phenanthroline should be mentioned here, for it has frequently given us good service in cases where it was not possible to obtain much blood or serum. The method is as follows:

Residual iron in serum, according to Kühnau. To 1 cc. of serum is added 1 cc. of distilled water and 2 cc. of 20% trichlor-acetic acid (distilled). Filter; to 3 cc. of the filtrate add 0.2 cc. of a 20% solution of cystein-chloral hydrate (Kahlbaum), 0.5 cc. of a solution of 0.05% *o*-phenanthroline-chloral hydrate, 0.5 cc. of a saturated solution of sodium carbonate, 0.5 cc. of a 20% solution of sodium cyanide, and 1.2 g. of sodium chlorate in powdered form. Shake until the solution becomes clear, then add 2 cc. of chloroform. Shake for 3 minutes and allow to stand for 10 minutes. Place the chloroform in a basin of a photometer (Pulfrich or Leifo) and read off the absorption with filter 600.

(c) *Criticism of the Quantitative Determination of Non-Haemoglobin Iron and Description of Four Fractions Characterised by Definite Physico-Chemical Properties*

While studying iron metabolism numerous clinical and experimental observations have shown us that the commonly applied methods of determining iron, particularly those of *Barkan* and *Heilmeyer*, are not without drawbacks involving errors which may sometimes be considerable. We wish to state here the reasons which have induced us to change these methods of estimation and to seek another method which would enable us to subdivide the non-haemoglobin iron into various fractions on the basis of their definite physico-chemical properties.

In making biological determinations of small amounts of iron the aim is to form a coloured iron complex. Colorimetric measurement of colour intensity permits of a quantitative determination. *Barkan* uses the iron-thiocyanate complex, whilst *Heilmeyer* prefers the iron-phenanthrolin complex. An important condition of these determinations is to establish a definite valency for the total quantity of iron to be determined. In the *Barkan* method the iron must be brought by oxidation to valency III, in *Heilmeyer's* method by reduction to valency II. However, from a practical point of view it is easier to reduce than to oxidise quantitatively. The 2% hydroquinone solution used by *Heilmeyer* does not always suffice to convert Fe^{+++} completely into

Fe^{++}. He considers that this is proved by the effect of an energetic reduction on the solution to be determined (as for instance, by the formation of hydrogen).

Example:

(1) Heilmeyer determination (Reduction by hydroquinone): 95 γ%
„ „ (But reduction by hydrogen): 125 γ%
(2) Heilmeyer determination (Reduction by hydroquinone): 110 γ%
„ „ (But reduction by hydrogen): 140 γ%

We will now state the most important arguments which have induced us to give preference to the iron-thiocyanate complex method of colouring.

The Most Important Characteristic Properties of Iron Determination According to the Phenanthroline Method

(1) Phenanthroline gives a colour reaction with the cation Fe^{++} of all ionised salts. Thus it suffices to add a powerful acid and a reducer in order to make colorimetry applicable to all the iron of the easily split-off complexes.

(2) The iron-phenanthroline complex cannot be extracted without a change of colour; hence colorimetry in the case of phenanthroline can only be applied to the clear solutions. But very often this condition cannot be realised in biological work. In such cases, despite intensive and protracted centrifuging, resort must be had to filtration, which by retaining the iron sometimes constitutes a source of error, as *Schmidt* and we ourselves were able to note. On the other hand, the determination of a solution stained prior to the addition of phenanthroline requires that the coefficient of extinction be determined both before and after the addition of phenanthroline. But this is usually not the case in determining the iron content according to the thiocyanate method in which the coloured iron-rhodanate is preferentially extracted by the ether, as will be shown.

Examples:

Standard iron solution of 129.5 γ%, titrated according to *Heilmeyer's* method (from Schmidt):

Without filtration	127.0%	127.0%	127.5%	127.5%
With filtration	70.2%	70.2%	70.5%	70.5%

Here are a few results of our own analyses in determining the iron in serum:

(*a*) Without filtration (pre-treatment with 6N HCl) 93%
With filtration (pre-treatment with 6N HCl) 55%
(*b*) Without filtration (pre-treatment with 6N HCl) 45%
With filtration (pre-treatment with 6N HCl) 10%

(3) Phenanthroline reacts only with Fe^{++}; but it is more difficult to reduce than to oxidise completely the iron contained in

biological material. This fact is of special importance in the analysis of serum and serum extracts after incineration in the air, where all iron is oxidised. On the other hand, under certain conditions, as a result of incineration, the oxide Fe_3O_4 may be formed which, in order to be ionised, needs the addition of HNO_3. Any further complete reduction of the hydroquinone will be a matter of practical impossibility.

(4) According to *Vahlquist*, maximal colour development necessitates a very exact pH, 1.65 to 1.95. Moreover, the quantity of H_2SO_4, which must be added after neutralisation by the *Heilmeyer* method, is a very important factor (*Schmidt*) and one that is not easy to carry out from a technical point of view.

Heilmeyer's technique demands an excess of exactly three drops N/2 H_2SO_4 after neutralisation with para-nitrophenol as indicator. The following are the results of Schmidt's analyses of a solution containing 121.5 γ% of iron:

Excess of N/2 H_2SO_4	Values obtained in γ%
0.005	78.0
0.020	99.0
0.040	112.0
0.060	122.0
0.090	123.0
0.110	120.0
0.130	118.0
0.180	97.5
0.220	67.5

We give below some of the values we obtained in determining the iron in the same serum according to *Heilmeyer's* original method (but without filtration); next according to *Schmidt's* original method, and finally according to *Schmidt's* method—with, however, reduction by hydrogen; these illustrate the above-described fluctuations of the iron values:

	Example 1	Example 2	Example 3
Heilmeyer-Hydroquinone	95 γ%	100 γ%	100 γ%
Schmidt-Hydroquinone	120 γ%	122 γ%	140 γ%
Schmidt-Hydrogen	125 γ%	124 γ%	150 γ%

(5) The colour product is stable. The maximal development of colour is, however, subject to periodic fluctuations, including those of temperature and the concentration of H_2SO_4. The photometer must not be read until twenty minutes after the colour has appeared.

(6) The presence of phosphates has no influence on the intensity of the reaction.

(7) The colour intensity of the Phenanthroline method is very great; it is usually the same as that of the Thiocyanate method.

The Most Important Characteristics of Iron Determination According to the Potassium Thiocyanate Method

(1) It acts only with the cation Fe^{+++} of iron chloride. It is easy to convert into $FeCl_3$ all the iron of the separable compounds and of the FeO and Fe_2O_3-oxides by the addition of HCl and an oxidising body, thus rendering it available for colorimetric examination.

(2) The iron-thiocyanate complex can easily be extracted from the usual biological solutions of ether, acetone and amyl-alcohol, without its colour undergoing any marked change. Therefore the technique can even be applied to turbid or discoloured solutions after simple centrifuging, and there is no need for filtration, which is apt to involve the above-mentioned errors.

(3) Thiocyanate reacts only with iron-chloride; but it is easier to oxidise completely than to reduce it completely. By the addition of a potassium solution to the solution to be determined any secondary reduction can be averted (*Breuer* and *Militzer*).

(4) For the maximal development of colour a highly acid medium is required (of pH below 2)—a condition which is automatically provided by the conditions of analysis. In a highly acid medium the thiocyanate is able to dissolve slowly and to form H_2S which reduces the iron, thus preventing colour formation. In order to avoid this difficulty it suffices to add a large excess of thiocyanate and of an oxidising agent, such as H_2O_2 (*Barkan*) or, better still, potassium persulphate (*Breuer and Militzer*). For the incineration persulphate is definitely preferable to hydrogen-peroxide.

(5) The colour of the iron-thiocyanate reaction may change in time. As the colour develops instantaneously, as soon as the potassium thiocyanate is associated with the $FeCl_3$, the reading must be taken immediately.

(6) The presence of phosphates prevents a certain amount of the iron from being determined quantitatively. This fact is of importance in connection with tissues rich in phosphates, such as the muscles and bone tissues, but it is of very slight importance in determinations of serum and serum extracts. According to *Leeper* the orthophosphates disturb the reaction but slightly, but the pyrophosphates do so to a greater extent. This source of error can be considerably diminished by thiocyanate in great excess. Thus *MacFarlane* and *Douglas* found that no calculable errors in their quantitative analyses of organ extracts were associated with phosphates. We have endeavoured to explain the role played by the presence of phosphates in serum determination by the thiocyanate method. A comparison of determinations conducted in parallel by the two colorimetric methods gives us, on the one hand, very similar

values for the direct determinations; on the other hand, almost identical relations between the results obtained before and after incineration.

From the above the following conculsions are drawn:

The chief disadvantage of the thiocyanate method lies in the fact that if certain substances are present, particularly phosphates, this method of determination gives low values. Nevertheless this drawback is practically negligible in the determination of iron in the serum and serum extracts—as proved by the results obtained in our parallel experiments before and after incineration with phenanthroline and thiocyanate. On the other hand, the thiocyanate method itself can also be applied to turbid and discoloured solutions, without involving the need of filtration, which is not always the case with phenanthroline. Finally, the thiocyanate technique is less complicated than that of phenanthroline.

Finally we tested the two methods by adding to one and the same serum a known quantity of iron and determining the iron content by both methods before and after the addition of iron. We obtained the following results:

6N HCl extract

Direct determination.	1st determination		2nd determination		
Thiocyanate method	160 γ%	add 180 γ%	350 γ%	recovered	190
Phenanthroline method					
of Heilmeyer	152 „	„ „ „	310 „	„	158
of Schmidt	180 „	„ „ „	350 „	„	165
of Schmidt, H_2 formation	185 „	„ „ „	370 „	„	180

6N HCl extract

Determination after incineration	1st determination		2nd determination		
Thiocyanate method	155 γ%	add 180 γ%	330 γ%	recovered	175
Phenanthroline method					
of Heilmeyer	150 „	„ „ „	315 „	„	165
of Schmidt	170 „	„ „ „	340 „	„	170
of Schmidt, H_2 formation	180 „	„ „ „	358 „	„	178

If we make iron determinations on one and the same serum according to the methods of *Barkan and Heilmeyer* we can see that the results are very different. This is probably due to the fact that the serum treated by the *Barkan* method was pre-treated with 1.2% HCl for one hour whereas that treated by the *Heilmeyer* method with a concentrated HCl solution (6N HCl) received previous treatment for only ten minutes.

Examples:

		Example A	Example B
Extraction 1.2% HCl read according to	Barkan	125%	115%
1.2% „ „ „ „	Heilmeyer	115%	100%
6N „ „ „ „	Barkan	160%	140%
6N „ „ „ „	Heilmeyer	120%	75%

As a general rule therefore the 6N HCl extracts more iron from the serum than does the 1.2% HCl. Thus the concentrated acid dissolves and ionises the serum iron more rapidly and completely —a condition which is indispensible for the colour reaction. Concentrations of HCl which are above 6N give lower iron values. This fact is probably partly due to the immediate and greater precipitation of the proteins by the concentrated acid. In a medium very rich in protein, such as the blood, extraction with 6N HCl yields less iron than does extraction with 1.2% HCl, as the last-named acid causes no protein precipitation.

	Extraction 1.2% HCl	Extraction 6N HCl
Blood ..	478 γ%	360 γ%

Immediate coagulation of the proteins probably takes with it part of the iron, which is thus withdrawn from the action of acid.

These considerations have led us to determine the aqueous extracts of the serum, i.e. the extracts obtained by the simple dilution of the serum with doubly-distilled water without the addition of HCl (before protein precipitation by trichlor-acetic acid). The values thus obtained correspond to iron which is chemically only loosely bound and which can be split off by a weak acid (trichlor-acetic acid).

		Direct Reading	Reading after incineration
Aqueous extracts	Serum 1	89 γ%	325 γ%
	„ 2	33 γ%	190 γ%
	„ 3	8 γ%	120 γ%
	„ 4	33 γ%	55 γ%
	„ 5	90 γ%	206 γ%
Extracts with 6N HCl	Serum 1	151 γ%	410 γ%
	„ 2	90 γ%	400 γ%
	„ 3	41 γ%	420 γ%
	„ 4	72 γ%	220 γ%
	„ 5	185 γ%	440 γ%

The question now remained whether the colorimetric methods hitherto applied permitted us to determine the iron in our extracts quantitatively with HCl, or whether they still contained iron in a form which could not combine with thiocyanate or phenanthroline; or if possibly the serum contained certain substances which might definitely influence the colour intensity of the iron reaction with thiocyanate or phenanthroline, thus falsifying the results of the determination. For this reason we incinerated the HCl extracts of the serum, the iron content of which had previously been calcu-

lated according to the methods of *Barkan and Heilmeyer*, the ash was dissolved and brought to its initial volume and the iron was determined by means of the same colorimetric methods. Above we give the results of these parallel determinations by the thiocyanate method for five different sera.

The extracts always or nearly always contained more iron than was obtained by the direct thiocyanate or phenanthroline determinations. In order to explain this fact we draw attention to the observations of *Starkenstein* and *Weden* relative to the presence of various fractions of iron in the blood and tissues. These authors found iron was present in the blood and organs in various forms soluble in HCl. The water extract of the blood and tissues, i.e. the extract that is obtained by the addition of water and subsequent precipitation of the protein bodies by trichlor-acetic acid, already contains small quantities of inorganic iron. With HCl greater quantities of iron can be extracted, although according to *Starkenstein* the total amount of inorganic iron can only be extracted after boiling it in 5N HCl, during which process the iron of the haemoglobin is not freed. In our own case, however, we were unable to note any increase of iron in the blood serum as a result of boiling the 5N extract, as can be seen in this table:

	Extraction by 5N HCl and boiling	Extraction by 6N HCl without boiling
Example 1	98 γ%	110 γ%
Example 2	117 γ%	130 γ%

Starkenstein and Weden distinguish three forms of inorganic iron in the blood:

(1) A very slight quantity of water-soluble, easily oxidisable divalent iron, "biologically active", which would correspond to the inorganic iron that has just been absorbed. This iron is slowly oxidised in the circulating blood, producing:

(2) Trivalent, water-soluble iron, which constitutes the major part of inorganic blood iron. As this fraction is difficult to reduce it may play the chief role in iron metabolism. Within the organism in the lung:

(3) It is reduced to divalent, inactive iron which is only soluble in concentrated HCl. This fraction must be considered the final product of iron metabolism. It is either eliminated by the organism or deposited in the spleen or liver. But these two organs are unable to take up and to fix the active divalent and trivalent iron.

The observations of *Starkenstein and Weden*, as well as our own findings, show the presence in the serum of a very easily separable iron fraction, which is only lightly bound, exists in an inorganic form and can be dissolved by trichlor-acetic acid after the precipitation of the serum protein bodies. *Vahlquist's* analyses by electro-

phoresis and dialysis show that the serum iron is completely bound up with the proteins of the serum; not only with the globulins but also, although to a lesser extent, with the albumins (30 to 50%). The serum iron cannot be dialysed at a pH of 10 to 4.5; between 4.0 and 3.5 dialysis enables about 50% of the serum iron to be separated; but even at pH 1.45 the dialysis is not complete.

The mineral acids can separate iron from its complexes; but trichlor-acetic acid, even in very strong concentration, is unable to split off the serum iron completely (*Fowweather*). Hence iron can be much more easily detached from its complexes by inorganic than by organic acids. On the other hand, *Vahlquist* has shown that the separation of the serum iron does not depend alone upon the pH of the acids used, but also upon certain characteristic properties of the serum. This fact which we also have repeatedly noted is similarly confirmed by *Moore* and his collaborators, who found unmistakable quantitative differences in the intensity of iron retention as a result of protein precipitation, according to the sera used.

We observed above that the colorimetric thiocyanate method does not enable us to determine the total quantity of iron extracted by means of HCl; it affects only that fraction which is split off by the acid. Hence we must assume that in addition there is another iron fraction which, however, remains in solution without being precipitated with the serum protein bodies and which, chemically, appears to belong to the group of complexes which are soluble in organic acids, but cannot be split off by HCl. Finally, one remaining iron fraction completely resists extraction by HCl. It can only be estimated when the serum which has not been previously treated is incinerated.

In this way the various methods of extraction enable us to differentiate four iron fractions, the biological significance of which will be defined in the course of our clinical and experimental observations.

Can iron that has been extracted by means of serum dilution with doubly-distilled water and subsequent protein precipitation by trichlor-acetic acid represent iron that is present in the serum in an ionised form? The investigations of *Vahlquist*, based on dialysis and electrophoresis, do not support this hypothesis. This author assumes that all the iron of the serum is bound up in complexes.

By means of ultra-filtration of the serum diluted with doubly-distilled water we obtain an aqueous extract that is completely iron-free, whilst ultra-filtration of the same serum, which has previously been treated by trichlor-acetic acid, yields an iron-containing extract. If we add to the serum small amounts of an aqueous iron-

choride solution it is possible to demonstrate the presence of iron in the ultra-filtrate; but the quantity of this metal that passes through the ultra-filter gradually diminishes, in proportion to the length of time that the iron solution remains in contact with the serum. Part of the iron becomes associated to and retained by the serum protein bodies, either by adsorption or by the formation of complexes. From this we must conclude that the serum contains no free iron in the ionised state.

Thus the serum iron in its totality is bound up in complexes. The addition of acids to the serum permits the iron to be liberated from certain complexes. Trichlor-acetic acid separates in this way a first weak iron fraction, which must be considered as loosely bound up with the complexes. Hydrochloric acid (in the concentration of 6N) usually separates the maximal part of the iron that can be split off by acids. The incineration of the HCl extracts separates a third iron fraction; this cannot be detected by direct colorimetry of the HCl extract that has not been reduced to ash; and this might be explained by the fact either that this fraction cannot be split off by HCl or that certain special substances, which have been destroyed by incineration, impede by their presence the colorimetric reaction by the thiocyanate or phenanthroline methods. Finally, a last-remaining fraction is precipitated with the serum protein bodies.

Accordingly we can distinguish four forms of serum iron:

Iron Fraction A = Easily split-off complex iron (Loosely bound Iron)

This iron is obtained by dilution of the serum with doubly-distilled water and subsequent protein removal with trichlor-acetic acid, the presence of which lowers the pH of the medium and renders possible the separation of this iron fraction. As this iron can easily be split off, this fraction is of great biological significance. Above all, it possesses the power of co-operating in the chemical exchanges of the organism. Thus the " active " inorganic iron of *Starkenstein* belongs to this fraction, and it would therefore participate chiefly in the biocatalytic processes of the living cell.

Iron Fraction B = Complex Iron difficult to split off (Firmly bound Iron)

This iron can only be split off by the addition of HCl. The extraction is dependent upon the concentration of the acid, but in part also upon the nature and quantity of the serum proteins. If the latter are precipitated, they remove a certain part of the iron from quantitative iron determination. Although much less easily separated than the iron fraction A, this fraction, which would

roughly correspond to *Starkenstein's* inorganic iron, must be considered to still partake very actively in the general iron metabolism.

Iron Fraction C = Iron of the acid-soluble Complex which cannot be split off

This fraction can only be quantitatively estimated after incineration of the HCl extract. The iron is not associated with precipitated iron; it cannot be split off by HCl. The possibility is not excluded that this fraction represents a part of the iron that could not be made visible in the B fraction, owing to the presence of definite substances which impeded the colorimetric iron reaction (phosphates). Moreover, we must note here certain organic compounds with iron (iron complexes) which cannot be shown in fraction B (see page 70).

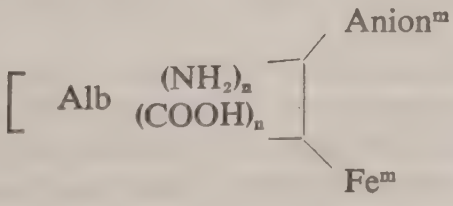
Iron Fraction D = Iron of the Protein Precipitate

This fraction completely resists extraction by HCl and can only be obtained by total incineration of the untreated serum. From this total iron the sum of the fractions A, B and C must be deducted. Probably this iron is either chemically bound in the interior of the protein molecules or physically by co-ordination-association, or by adsorption, or finally it may be incorporated in the protein precipitate. For instance, this fraction includes the iron of haemoglobin which is precipitated with the protein as a result of the presence of globin.

The presence of haemoglobin iron in this fraction often leads to great quantitative fluctuations of the D-iron. Actually slight haemolysis of the blood is sufficient to provoke a marked increase of the serum-haemoglobin content and consequently of the iron content. For this reason this fraction is the least constant and, biologically considered, the least important. Marked increase of the D-iron is usually a sign of haemolysis or hyper-proteinaemia.

Owing to the complexity of the factors and processes involved it is impossible to avoid classifying the designations and characteristics of our four iron fractions.

In this connection it may be well briefly to draw attention to the findings of *Bechhold*, who noted the presence in protein and iron-salt solutions of a number of complexes which show more or less resistence to HCl action and in which the iron is bound up with the protein bodies according to the following design:



Technique of the Determination of Iron and its Four Fractions in the Serum

Reagents.

(1) Doubly-distilled water.

(2) 25% trichlor-acetic acid (prepared from re-crystallised trichlor-acetic-acid Merck and doubly-distilled water).

(3) 6N HCl (prepared from HCl A.R. Merck or Schering-Kahlbaum 37%).

(4) 20% KCNS A.R.

(5) Peroxide-ether, freshly prepared as follows: Place in a separating-funnel 0.5 cc. of a 0.03% solution of H_2O_2 (freshly prepared with doubly-distilled water and Merck Perhydrol 30%) and 25 cc. of anaesthetic ether. Shake vigorously. Allow the water to settle to the bottom of the funnel.

(6) N/1 H_2SO_4 A.R. Merck.

(7) 17% NH_4OH A.R. Merck.

(8) 20% potassium persulphate, A.R.

Preparation of the Glassware. The clean glassware is left from eight to ten hours in a mixture of chromic-sulphuric acid (400 g. $K_2Cr_2O_7$ to 5 litres of distilled water and 3 litres of H_2SO_4, concentrated, sp. gr. 1.84). Next it is thoroughly washed in distilled water and finally well rinsed four times with doubly-distilled water.

Determination of Iron Fraction A. Add to 4 cc. of serum 2 cc. of doubly-distilled water. Mix well with a glass rod; allow to stand for fifteen minutes. Add 2 cc. of 25% trichlor-acetic acid; shake, stand for fifteen minutes. Centrifuge, decant. To 3 cc. of the extract add 2 cc. of 20% KCNS, then 3 cc. of peroxide-ether. Seal test-tube and invert it four or five times. After two minutes shake it energetically while inverting the test-tube ten times. As soon as the liquids are separated read the adsorption by the photometer with filter 510. A standard curve which is set up with a solution of $FeCl_3$ of known concentration permits the extinction to be converted directly into gamma of iron in 100 cc. of serum. In doing this the value of the blank, which should be done for each iron determination, must be deducted.

It is highly important that the photometric reading be made quickly, immediately after shaking, in order to prevent the formation of a yellow precipitate which would gradually pass into the ether layer and finally change the colour or produce a Tyndall phenomenon, which represents another common source of error in photometric readings.

Determination of Iron Fraction B. To 4 cc. of serum add 2 cc. of 6N HCl; mix with a glass rod and stand for 15 minutes; add 2 cc. of 25% trichlor-acetic acid; mix; stand for fifteen minutes. Centrifuge, decant; make photometric determination as for Iron

A. From the values obtained the value of Iron A must be deducted.

Determination of Iron Fraction C. Mix in a platinum crucible 3 cc. of the B extract obtained according to the method of Fraction B, which has been centrifuged and decanted, with 2 cc. of H_2SO_4 normal solution. Evaporate at 100–120° (about twelve hours). Then add 2 cc. of 17% NH_4OH; mix, evaporate at about 140° (about six hours). Incinerate slowly with a small, slightly oxidising flame. The crucible must not exceed a red glow (dark red colour). It sometimes happens that certain organic particles must be spread out at the bottom of the crucible in order to be completely incinerated. For this purpose 1–2 drops of doubly-distilled water are added; it is evaporated and the incineration is continued. (Hydrogen-peroxide must not be used to accelerate the destruction of the organic substances; for the addition of Perhydrol often causes loss of iron, either by bursting the vessel, which may occur even in the cold, or by facilitating the formation of a black sediment, which would be almost insoluble in H_2SO_4.)

When the crucible contains nothing more than a homogeneous fine ash the latter is taken up with 2 cc. of a normal solution of H_2SO_4 and the mixture is left to stand for two hours. Add 2 cc. of doubly-distilled water and stand for one hour. Pour the contents of the crucible into a test-tube, rinse the crucible with 2 cc. of 20% KCNS and add the contents of test-tube. Add 1 drop of 20% potassium-persulphate, plus 1 cc. of 6N HCl, and finally 3 cc. peroxide-ether. Seal, shake and read by photometer, as above. Deduct from value obtained the value of the blank test and the sum of Fractions A and B.

Determination of Iron D and of Total Iron. Mix in a platinum crucible 1 cc. of serum with 1 cc. of 6N HCl; after five minutes add 2 cc. of N/1 H_2SO_4; mix; evaporate at 100–120° (twelve hours). Add 2cc. of 17% NH_4OH; mix; evaporate at 120–140° (six hours). Incinerate. Take up the ash as described above and determine Fe photometrically. Deduct the value of the blank test. Divide the result obtained by 2, as the serum here is undiluted, and multiply by 3, owing to the dilution of the iron of 1 cc. of serum in 3 cc. of ether. Deduct from the result the sum of Fractions A, B and C.

It must be well borne in mind that in cases of haemolysed serum the result obtained may sometimes be too low; the more haemoglobin is present the greater will the deficiency appear to be. This error can be considerably reduced by repeating the incineration; that is, by taking up the ash of the first incineration with H_2SO_4, evaporating it, adding NH_4OH, evaporating again and incinerating it a second time. Only then is the ash to be taken up for determination. In such a case the blank test must of course contain twice as

much H_2SO_4 and NH_4OH, as was the case above. For the exact method of testing compare the original work of J. J. Schenk (*Schweiz. med Woch.*, 1944, 64).

The calculation of the four fractions is made in the following manner:

Iron Fraction A = Iron directly calculated according to the above-described method of extraction.
Iron Fraction B = Iron calculated according to the described method of extraction – Iron A .
Iron Fraction C = Iron from the incineration of the hydrochloric acid extract – Iron A and B.
Iron Fraction D = Iron of the direct serum incineration – Iron A, B and C.
Total Iron = Iron of the direct serum incineration.

Proceeding from these fractions we have endeavoured to study systematically the qualitative distribution of serum iron in healthy individuals, and to follow the possible variations which exist between normal men and women.

The results of these investigations are summarised in the following table.

Non-Haemoglobin-Iron of the Serum and its Four Fractions in Healthy Men

Age	Iron Fraction A	B	C	D	Total Iron
Mié. 22 years	15 γ%	165 γ%	70 γ%	20 γ%	270 γ%
Mar. 23 „	20 γ%	200 γ%	60 γ%	20 γ%	260 γ%
Dav. 24 „	40 γ%	120 γ%	40 γ%	60 γ%	260 γ%
Bou. 25 „	10 γ%	175 γ%	10 γ%	80 γ%	275 γ%
Pah. 26 „	10 γ%	150 γ%	90 γ%	60 γ%	310 γ%
Cra. 27 „	40 γ%	160 γ%	70 γ%	25 γ%	295 γ%
Lic. 28 „	45 γ%	115 γ%	140 γ%	45 γ%	345 γ%
Sch. 28 „	5 γ%	115 γ%	50 γ%	50 γ%	230 γ%
Boc. 29 „	45 γ%	155 γ%	50 γ%	45 γ%	295 γ%
Sol. 29 „	15 γ%	165 γ%	140 γ%	55 γ%	375 γ%
Del. 30 „	10 γ%	135 γ%	35 γ%	50 γ%	230 γ%
Reg. 40 „	5 γ%	130 γ%	135 γ%	60 γ%	330 γ%
Sic. 50 „	10 γ%	190 γ%	100 γ%	30 γ%	330 γ%
Average values:	21 γ%	152 γ%	76 γ%	46 γ%	296 γ%
Calculation of Fractions in % of Total Iron	7%	51%	26%	16%	

Non-Haemoglobin-Iron of the Serum and its Four Fractions in Healthy Women

Age	Iron Fraction A	B	C	D	Total Iron
Ahe. 18 years	0 γ%	115 γ%	15 γ%	25 γ%	155 γ%
Cha. 20 „	0 γ%	140 γ%	110 γ%	35 γ%	285 γ%
Kob. 21 „	0 γ%	80 γ%	50 γ%	40 γ%	70 γ%
Mar. 23 „	10 γ%	150 γ%	55 γ%	35 γ%	250 γ%
May. 24 „	10 γ%	70 γ%	145 γ%	45 γ%	270 γ%
Dum. 26 „	10 γ%	155 γ%	45 γ%	55 γ%	265 γ%
Her 28 „	10 γ%	175 γ%	0 γ%	45 γ%	230 γ%
Map. 29 „	10 γ%	140 γ%	40 γ%	55 γ%	245 γ%
Nic. 36 „	10 γ%	150 γ%	80 γ%	40 γ%	280 γ%
Duv. 39 „	5 γ%	175 γ%	70 γ%	25 γ%	275 γ%
Average values:	6 γ%	135 γ%	61 γ%	40 γ%	242 γ%
Calculation of Fractions in % of Total Iron	2%	56%	25%	17%	

These observations, which were made on individuals who were in excellent health and who were shown to possess a good general constitution, permit us to draw certain conclusions of general interest.

In the first place, we note that the total iron content and its four fractions may vary as between one individual and another. Serum iron has no absolutely constant value, as would appear from the observations of *Heilmeyer* and *Plötner*; on the contrary, it is frequently subject to considerable fluctuation, as *Vahlquist* has also recently shown. Actually, according to *Vahlquist*, serum iron varies in normal individuals between 68 γ% and 263 γ%.

Recently *Knud Hoyer* made a number of interesting observations regarding the physiological fluctuations of the serum iron content in human beings. In determinations regularly conducted once a week variations in the iron content can be observed which in men may amount to 94.5 γ% and in women to 89.3 γ%. Moreover, important differences and fluctuations may also occur from day to day. If, finally, the daily differences in the serum iron are determined, a typical serum iron curve can regularly be observed in normal human subjects, illustrative of a reduction of serum iron content from morning to evening and a corresponding rise from evening to morning.

This fact was also determined by *Hemmeler*. This reduction of the serum iron during the day is probably dependent upon the muscular activity of the organism. (For mobilisation of the

iron during muscular exertion, according to *Vannotti*, see below.) As a matter of fact, the daily curve for individuals who work at night shows a course the reverse of that of day workers. *Hemmeler* is of the opinion that certain neuro-vegetative influences also play a part in this daily fluctuation.

It is not possible to institute an exact comparison between *Barkan's* normal iron values, *Heilmeyer's* iron and our own fractions; for, as we have previously emphasised, the iron quantities thus obtained are dependent upon the methods of determination.

Our Fraction A + B is the one that comes closest to *Heilmeyer's* fraction. It corresponds to the iron isolated by the 6N hydrochloric acid, as does *Heilmeyer's* fraction; but, as has been emphasised, the duration of the action of the acid on the serum is longer in the case of Iron B than in *Heilmeyer's* method. The B-Iron is coloured by thiocyanate and not by phenanthroline. Finally, we have also eliminated the disadvantage connected with filtration, which always retains some iron. This explains why the values of A + B-Iron are higher than those of *Heilmeyer's* serum iron. *But from a practical point of view these two iron values are similar. They represent the important elements for the clinical study of Iron Metabolism.*

We give below the average values of serum iron, according to the method of *Heilmeyer*, as described in the most important works dealing with the problem of serum iron and which *Vahlquist* takes as a basis.

	No. of cases	Men	Women
Heilmeyer and Plötner, 1937	25	126.2	88.5
Moore and collaborators, 1937	15	121.5	97.6
Skouge, 1939	50	117.7	104.4
Brochner-Mortensen and Olsen, 1940 ..	20	131.9	127.0
Vahlquist, 1941..	50	142.0	123.0
Average value	160	127.8	108.1

The average value of our B-iron fractions clearly approximates the values obtained by *Vahlquist*. The difference between men and women is + 17 for B-iron (+ 19, *Vahlquist*); but according to *Heilmeyer* and *Plötner* it is + 37.7 for serum iron.

Fraction C requires to be studied with precision. At first, one might think that the hydrochloric acid would dissolve all the iron contained in the serum which is not joined to the molecule of the haemoglobin. However, exact determinations by ashing, avoiding possible errors due to penetration of iron during the ashing and to the iron contents of the reagents, showed that almost always the ash of the HCl extract contains little iron that was not revealed by the direct colorimetric method with thiocyanate.

We have already mentioned the formation of complexes of phosphates with iron causing errors in determination which can be important if there is a high percentage of phosphorus (*Elvehjiem* and his collaborators). These errors are not important with our method, because we work with a rather low pH. But there are other iron complexes which can thus mask the presence of iron. *Jackson* speaks, for instance, of complexes produced by protein hydrolysates. *Treadwell* speaks of iron complexes with the organic oxy-acids (tartaric acid and citric acid), with glycerine, and various sugars. Oxalic acid must also be taken into consideration. Lastly, *Smythe* and *Schmidt* described complexes of ferric iron with amino acids which cannot be detected with thiocyanate, if the amino acid forms an iron compound less dissociated than ferric thiocyanate.

By ashing, it is probable that the greater part of these complexes are destroyed and that we thus succeed in liberating the iron present (see *Chatelan* and *Senn*).

It seemed of interest to follow the variations of the different fractions of non-haemoglobin iron after oral or intravenous administration of iron.

Here are a few examples:

1. Peroral administration of 1 g. of ferrum reductum with simultaneous intake of 1 tablet of Acidol-Pepsin in a case of anaemia:

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	0 γ%	120 γ%	90 γ%	80 γ%	270 γ%
3 hours after iron intake ..	0 γ%	210 γ%	110 γ%	50 γ%	370 γ%
6 hours after iron intake ..	0 γ%	185 γ%	350 γ%	50 γ%	585 γ%

2. Administration per os of 1 g. ferrum reductum:

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	20 γ%	240 γ%	100 γ%	35 γ%	395 γ%
3 hours after iron intake ..	60 γ%	400 γ%	120 γ%	40 γ%	620 γ%

3. Administration per os of 1 g. ferrum reductum:

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	40 γ%	35 γ%	75 γ%	350 γ%	500 γ%
3 hours after iron intake ..	100 γ%	140 γ%	110 γ%	200 γ%	550 γ%
6 hours after iron intake ..	170 γ%	80 γ%	300 γ%	260 γ%	810 γ%

In this case there was pronounced haemolysis during the first determination, resulting in considerable increase of the D-iron and consequently also of the total iron.

As a general rule, therefore, peroral iron administration is followed by an increase of the separable iron of the complexes: First the loosely bound, then the closely bound fraction. Only much later, six hours after iron intake, is it possible to note a definite increase of the C-iron belonging to the non-separable complexes. On the other hand, the iron that is precipitated with the serum protein does not appear to take part in the increase.

All the above cases showed clinically a certain degree of anaemia; they needed iron and absorbed part of it, as could clearly be demonstrated in our determinations. Normal individuals, on the other hand, who are well supplied with iron, usually show as a result of oral administration only a minimal increase of serum iron. The intravenous injection of iron is also accompanied by a marked increase of the serum iron. In these experiments we used "Ce-Ferro" (3 cc. = 6 mg. Fe^{++}). Here are two examples:

1.	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	0 γ%	180 γ%	100 γ%	30 γ%	310 γ%
30 min. after injection ..	25 γ%	400 γ%	65 γ%	30 γ%	520 γ%
2 hours after injection ..	0 γ%	380 γ%	70 γ%	30 γ%	480 γ%
6 hours after injection ..	0 γ%	320 γ%	60 γ%	20 γ%	400 γ%

2.	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	30 γ%	140 γ%	90 γ%	60 γ%	320 γ%
30 min. after injection ..	30 γ%	370 γ%	50 γ%	50 γ%	500 γ%
4 hours after injection ..	30 γ%	320 γ%	40 γ%	40 γ%	430 γ%

These observations showed after intravenous injection the presence in the serum of a somewhat higher quantity of iron than might have been expected from theoretical calculations based on the distribution of 6,000 γ of iron in the total quantity of blood.

We wondered whether, as a result of the intravenous "Ce-Ferro" administration, reserves of iron might not have been mobilised, or slight haemolysis might not have been produced. "Ce-Ferro" is a divalent iron solution which is stabilised by ascorbic acid. Its pH is fairly low and might cause a certain degree of local haemolysis during injection. By means of its reducing effect the ascorbic acid solution might possibly also provoke a mobilisation of the reserve iron or of the peripheral iron in the blood, or even a change in the iron distribution of the iron fractions in the serum.

In order to clarify this point we conducted a series of experiments for the purpose of studying the serum iron changes following upon intravenous injection of weak concentrations of organic acid solutions of pH such as ascorbic acid. These are the results obtained:

Intravenous injection of 5 cc. of 1% acetic acid in a physiological salt solution:

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	0 γ%	200 γ%	100 γ%	25 γ%	320 γ%
20 min. after injection ..	0 γ%	200 γ%	70 γ%	20 γ%	300 γ%

Intravenous injection of 5 cc. of 1% citric acid in a physiological salt solution:

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	0 γ%	150 γ%	80 γ%	20 γ%	250 γ%
20 min. after injection ..	0 γ%	120 γ%	70 γ%	40 γ%	230 γ%

The fluctuations we found were slight; they were within the limits of error of the method of determination. There was a slight reduction of the total iron but a certain tendency for the D-iron to increase, which might indicate slight haemolysis as a result of the injection.

The intravenous administration of pure ascorbic acid gave the following results:

Intravenous injection of 1% of ascorbic acid solution:

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	5 γ%	200 γ%	50 γ%	100 γ%	355 γ%
20 min. after injection ..	10 γ%	195 γ%	65 γ%	80 γ%	355 γ%

Intravenous injection of 5 cc. of ascorbic acid solution:

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	0 γ%	210 γ%	50 γ%	80 γ%	340 γ%
20 min. after injection ..	0 γ%	230 γ%	65 γ%	50 γ%	345 γ%

The injection of the ascorbic acid similarly fails to provoke any significant change in the serum iron; but a tendency to an increase of the B- and C-iron after injection can sometimes be noted.

Finally we tried to follow the fluctuations of the serum iron following upon injection of Redoxon, i.e. a sodium salt of ascorbic acid which has the advantage of producing no acid reaction.

Intravenous injection of 5 cc. Redoxon (strong):

1st experiment

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	0 γ%	185 γ%	100 γ%	60 γ%	345 γ%
20 min. after injection ..	0 γ%	220 γ%	66 γ%	60 γ%	345 γ%
2 hours after injection ..	0 γ%	205 γ%	75 γ%	50 γ%	330 γ%

2nd experiment

	A-Iron	B-Iron	C-Iron	D-Iron	Total Iron
Before	0 γ%	155 γ%	115 γ%	100 γ%	370 γ%
20 min. after injection ..	0 γ%	175 γ%	100 γ%	95 γ%	370 γ%

The hypothesis that ascorbic acid is capable of slightly changing the distribution of the serum iron by increasing the content of the separable iron at the expense of the non-separable complexes thus appears to be confirmed.

Finally, we must not forget that Vitamin C exerts a favourable effect on iron absorption by the intestine—an effect that, according to *Lucksch* and *Rominger*, is attributable to an irritant action by this vitamin on the gastric secretion. An increase of iron, and particularly of the B-fraction, following upon ample administration of Vitamin C, might therefore also be explained by increased iron absorption by the intestinal tract, which occurs more especially a considerable time after administration. Finally, we consider it worth while to emphasise at this point the findings of *Schröder* and *Braun-Stappenbeck*, who noted in a number of patients a certain parallelism between the Vitamin C content and the serum iron content of the blood. This included the observation that repeated administration of Vitamin C produced increase of iron in the serum.

(d) Quantitative determination of iron with α, α′–dipyridyl

This method, recently described by different authors (see *Lange, Woiwod* and collaborators and others), has the great advantage of giving a stable coloration; it is very sensitive (less sensitive, however, than the thiocyanate method).

Procedure according *Lange*:

Mix 2 cc. serum and 1 cc. 6N HCl, and allow to stand for 10 minutes. Then add 2 cc. trichloracetic acid, filter. To 2.5 cc. of filtrate add 1 drop paranitrophenol (1% alcoholic solution), neutralise with conc. NH_3. Add 0.5 cc. 5% Na_2SO_3 + 1 cc. acetate buffer + 0.5 cc. 5% α, α′–dipyridyl. Add distilled water to a total volume of 5 cc. Wait 10 minutes before reading on the colorimeter.

V. Considerations Regarding the Physiology of Non-haemoglobin Iron

We mentioned above the differences of opinion provoked by the hypothesis of the easily split-off iron of *Barkan* and the arguments advanced by that author to prove that his iron fraction is not a product of the artificial decomposition of haemoglobin. To-day we know that the "easily split-off iron" of *Barkan* represents an iron fraction pre-formed in the blood and serum, corresponding to the iron which is loosely bound in complexes. There is even a fraction which can be loosened from the complexes by organic acids, such as trichlor-acetic acid. In whole blood the values of the easily split-off iron are ten to twelve times greater than in serum. As oral administration causes an increase of this iron in the whole blood in the same way as in the serum it is to be assumed that the iron which is freshly absorbed from the intestinal tract is about equally distributed between cells and serum and, in part at least, circulates in the whole blood in the form of easily split-off iron. The high content of this iron in the whole blood is, however, not only due to the supplies of freshly absorbed iron, but also to the presence of a considerable quantity of iron in the erythrocytes, the origin of which is not alimentary. There is no doubt that we are here concerned with iron of various origins and sources.

A first fraction is derived from the intestinal canal and corresponds to the reduced iron which *Starkenstein* designates as biologically active. A second fraction comes from the progressive oxidation of this reduced active iron and would accordingly be equivalent to the active trivalent iron of *Starkenstein*. According to this author the active divalent iron is fairly rapidly converted in the blood into trivalent iron. This is comparatively stable in the serum, but continues to be reduced in the whole blood, although slowly, into divalent iron, thus forming a third fraction of non-haemoglobin iron. This explains the fact that in the whole blood there is relatively much more non-haemoglobin iron than in the serum. The property possessed by Barkan's "easily split-off iron" of reversibly binding oxygen and carbonic oxide permits the assumption of a pseudo-haemoglobin and accordingly of a fourth iron fraction. This would be the expression of a slow decomposition of haemoglobin in the circulating blood, which is independent of the reticulum; it would occur primarily in the red blood cells.

The separable iron of the blood does not therefore represent in its totality an intermediary product of haemoglobin and bilirubin but a group of various fractions of different origin: active iron

derived from intestinal absorption; trivalent iron produced by oxidation of the reduced active iron; further, the iron proceeding from the slow decomposition of the haemoglobin circulating in the blood, and from many other fractions hard to define as, for instance, iron transported to the different tissues that need it and to the organs of storage. The iron originating from haemoglobin decomposition in the blood-stream and representing pseudo-haemoglobin appears to be almost exclusively bound up with the stroma of the erythrocytes. It has only a secondary connection with the serum iron.

From these considerations, as well as from a comparison of our own results with those of the corresponding statements found in the literature, we envisage normal iron metabolism as follows:

The iron proceeding from the intestinal canal and representing the nutritive iron is converted by the gastric juice into a reduced inorganic form. Absorption of ferrous iron occurs in the mucosal cells of the duodenum and jejunum by the mechanism of ferritin formation. (An iron-containing protein contains as much as 23% of iron.) Iron absorption is regulated by the equilibrium between ferritin, ferrous iron in the intestine, and non-haemoglobin iron in the plasma. The level of this circulating iron is fixed partly by the percentage and the nature of blood proteins. The plasma iron level also depends on the rate of absorption of iron from the intestine, on the need of iron for the tissues and principally for the bone-marrow; it depends too on the destruction of haemoglobin and on the quantity of iron which is in the storage organs. The liver plays an important part as an organ of elaboration, storage and excretion of circulating iron. The greater part of the circulating iron is normally used for the formation of haemoglobin; only a small part is deposited in the tissues or serves either as an element of cellular structure or as catalyst.

In conclusion mention must be made of *Barkan's* pseudo-haemoglobin, as the product of a slow decomposition of haemoglobin into bilirubin in the erythrocytes, as well as of a few iron-protein complexes (*Starkenstein's* ferro-globulins).

Among all the serum iron fractions hitherto described, *Heilmeyer's* serum iron, as we have found above, is not free of errors in its method of determination, nevertheless it retains its full value from a clinical-practical point of view; for it represents that iron fraction which is most important in estimating the physiological and pathological fluctuations of serum iron.

The sub-division into the four above-described iron fractions, on the basis of definite physico-chemical properties, is principally intended to serve the purpose of a practical clinical differentiation

of the total serum iron, based in the main on the separation of the iron complexes which are, respectively, easy and difficult to split off from the Fe of the non-separable complexes and from the one which is precipitated with the serum protein. In setting up these four fractions it is not our intention to indicate four sharply separated biological and exactly defined units of the total iron metabolism; we merely wish to follow the variations of these fractions, which are to a certain extent chemically separated, under normal and pathological conditions, thereby offering important criteria for judging the total iron metabolism.

The numerous clinical observations, which up to the present time have been based mainly on the work of *Barkan* and *Heilmeyer*, permit us to conclude that extraction by means of hydrochloric acid produces an iron fraction which is fairly stable and hence possesses a definite clinical value. In normal individuals this fraction shows clear variations as between men and women. From the observations of *Fowweather, Goidsenhoven* and collaborators, *Heilmeyer* and *Plötner, Moore* and collaborators, *Vahlquist, Vannotti* and others, it can be seen that the iron fraction extracted by 6N HCl shows, in accordance with the various authors and methods of determination, an average value of 125 to 142 $\gamma\%$ in healthy men and of 89 to 123 $\gamma\%$ in healthy women. According to our method of procedure the value of the iron fraction extracted by HCl is 152 $\gamma\%$ in men and 135 $\gamma\%$ in women.

The serum iron content in women has recently been the subject of a detailed clinical study by *Albers*. According to this author the serum iron content in women during the menstrual cycle shows no essential fluctuations, if menstruation is normal. The serum iron usually decreases during the menopause; from an average value of 91 $\gamma\%$ before menopause, it falls to an average of 76 $\gamma\%$ after. He is of the opinion that this decrease is caused by endocrine factors. Finally, we are interested to note that according to *Albers* the relatively low serum iron content in women is not caused by the periodic losses of blood, but is rather a quality associated with the sexual characteristics and hence a function of the endocrine system. When menstruation starts the iron content increases instead of diminishing. There is no essential difference between the iron content in men and women in the period before puberty. Only after the onset of puberty does the serum content increase in men.

During pregnancy the serum iron is invariably increased (average rise of 33%). These findings appear to confirm the experimental observations of *Kojima*. This author was able to show an iron impoverishment of the other organs during pregnancy in favour of the uterus, the iron supply of which becomes considerably enriched. Here, therefore, we see a mobilisation of all the mother's

deposit iron, for the purpose of giving up this metal to the foetus. This mechanism ceases immediately after birth.

In the foetal blood the iron content is the same as in the mother. According to *Vahlquist* and *Albers* a rapid decrease of the iron content can be observed in the newly-born during the first 24 hours after birth (of 160 γ% to 50 γ%)—a fact doubtless to be attributed to the circumstance that iron ceases to be given up through the mother's blood; in our opinion it may possibly also be attributable to the profound and fundamentally altered conditions of circulation and breathing associated with birth. During the first two weeks a gradual increase to about 125 γ% can be noted, whilst in the course of the first two years a decrease of about 60 γ% in the physiological serum iron can be observed. But this does not justify *Vahlquist's* assumption that there is increased haemolysis during the first years of life.

It seems useful to note here the observations of *Vahlquist* and *Hemmler* on the daily variation of the percentage of non-haemoglobin iron. These variations present a certain rhythm which does not seem to be particularly influenced by the iron absorbed from food, but rather by the energy requirements of the organism and by certain variations of the tonus of the neuro-vegetative system. In fact, there is a decrease of iron during the day and an increase to the normal level during the night. This rhythm is inverted in persons who work during the night and rest during the day.

PART TWO

IRON METABOLISM UNDER PATHOLOGICAL CONDITIONS

We have briefly considered the problem of normal iron metabolism as it can be envisaged on the basis of the numerous clinical and experimental works which have hitherto been undertaken. It will now be our task to study the circulating non-haemoglobin iron in human pathology and in animal experimentation under various pathological conditions. We shall endeavour to show that the quantitative and qualitative determination of the circulating non-haemoglobin iron has become a useful and indispensable aid for the clinician. It permits of greater accuracy and completeness in the study of the functional state of the organs and systems and in judging their connection with iron metabolism.

In our investigations we made use of various methods of determining iron; in addition to the systems of *Barkan* and *Heilmeyer*, often simultaneously applied, we resorted chiefly to our method of fractional determination described above. The latter, in our opinion, offers a more complete picture of the changes occurring in the iron circulating in the serum in the course of various pathological conditions.

As has been emphasised at the end of Part One, we consider that these various fractions represent neither exactly defined biological units which are mutually excluded by the selected method of extraction, nor the expression of an artificially defined fraction; neither do we believe that they are an exact indication of their origin or biological value. We see in them merely iron fractions which can regularly be demonstrated as a result of quite definite physico-chemical methods of procedure. During the development of a pathological process these fractions often show characteristic fluctuations which in certain cases permit us to draw certain conclusions of biological and practical significance. By comparing the clinical or anatomo-pathological picture with the results of our determinations we have frequently been enabled to draw certain useful inferences from the study of iron metabolism under pathological conditions.

Our observations have been described in the following chapters, each one of which deals with a quite definite problem in the domain of changes occurring in iron metabolism during erythropoiesis, during the conversion of blood and tissue pigment, and during disturbances of the hepatic function and of general metabolism.

I. Iron and Blood Changes

A. *Haemolysis*

In haemolysis, in which the destruction of the red blood cells is accompanied by the liberation of a certain amount of blood pigment and its derivatives, the circulating plasma becomes enriched with iron, which in part belongs to the haemoglobin molecule, in part to the intermediary substances of blood decomposition. If, therefore, in the course of haemolysis and immediately afterwards the plasma is rich in haemoglobin iron this does not suffice to indicate that it has become richer in iron, which is more or less completely separated from the organic residual constituents of the haemoglobin.

As is well known, haemolysis is accompanied by increased elimination of urobilin; it is often followed by marked bilirubinaemia. Thus the organism would appear to convert the haemoglobin which has been freed by haemolysis by decomposing it into bilirubin and its iron-free derivatives. We must therefore assume that, by this means, iron is released and that, during a first phase, haemolysis increases the haemoglobin-iron fraction and, during a second phase, increases the non-haemoglobin-iron fraction. The essential point is to know whether this increase takes place only in the organs which are actively involved in haemoglobin conversion (spleen, liver, reticulo-endothelium) or whether it is also made evident in the circulating blood.

Observations of recent years furnish us with a rich documentation for deciding this problem. Actually an increase of the non-haemoglobin iron has often been noted (*Heilmeyer* and *Vannotti*), either in acute haemolysis (haemolytic icterus, haemoglobinuria) or in pernicious anaemia, in which there is a noticeable increase of haemolysis. The problem with which we are especially confronted is to determine the state of the various non-haemoglobin-iron fractions during haemolysis, particularly that of *Barkan's* " easily split-off iron ".

According to *Barkan* the " easily split-off iron " is a quite definite iron fraction which represents the intermediary phase in haemoglobin decomposition into bilirubin and which must not be confused with the total iron of the serum or plasma. If, therefore, *Barkan's* hypothesis is valid this non-haemoglobin-iron fraction should show unmistakable quantitative differences in the course of haemolysis. In view of the fact that valuable data regarding this point can be obtained by experiment, *Barkan* endeavoured to make quantitative determinations of the fluctuations of the iron fraction during phenylhydrazine haemolysis in rabbits. After a lengthy preparation with phenylhydrazine he noted, in three cases,

marked anaemia with, however, a simultaneous clear diminution of the " easily split-off iron ". In one case this fraction showed no change.

The experiments of *Barkan* are not conclusive. The decrease of iron, as *Heilmeyer* correctly states, can, on the one hand, be the result of the anaemia that has been produced, and, on the other hand, the expression of blood regeneration in the bone-marrow. *Barkan's* results are based on experiments with a chronic evolution; hence they are the expression of a diversity of changes in haemoglobin metabolism.

Heilmeyer cites the two following observations: The administration of 0.2 g. of phenylhydrazine daily for three days to normal individuals gave the following results for serum iron:

1st case:
134 γ% before phenylhydrazine, 317 γ% after phenylhydrazine.
2nd case:
122 γ% before phenylhydrazine, 216 γ% after phenylhydrazine.

Moore, Doan and *Arrowsmith* find in haemolysis a certain balance between the iron freed by the destruction of red blood corpuscles and the iron needed for blood regeneration. Splenectomy in haemolytic anaemia causes a reduction of the plasma iron. During haemolysis produced by phenylhydrazine, these authors noted a distinct increase of iron in the plasma; on the other hand, *Barkan's* " easily split-off iron " did not change.

Vahlquist, who injected rabbits intravenously with phenylhydrazine and distilled water, regularly noted a distinct increase of the serum iron as soon as fifteen minutes after a single injection (0.02 g. of phenylhydrazine per kg.). The maximal increase was attained from one to six hours after the injection. The values, which were above normal, were maintained for three to five days. After that, a fall of the serum iron content to below normal figures was regularly observed.

Before entering upon the systematic treatment of the problem we wish to report upon a few personal observations. In the rabbit we followed the content of the *Barkan*-iron in the blood and serum, as well as the " serum iron " of *Heilmeyer*, and finally the values

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Rabbit No. 1. First injection of 8 cc. of distilled water intravenously; second injection of 4 cc. of distilled water 32 hours after the first injection.

	Hb	Barkan Iron		Heilmeyer
		Blood	Serum	Serum Iron
		γ%	γ%	γ%
Before	70%	120	28	168
20 minutes after 1st injection ..		172	18	192
2 hours after 2nd injection ..		114	38	208
48 hours after 1st injection ..	45%	114	76	259

Rabbit No. 2. Injection of 6 cc. of distilled water intravenously.

	Hb	Barkan Iron Blood	Barkan Iron Serum	Heilmeyer Serum Iron
		γ%	γ%	γ%
Before haemolysis	75%	170	42	162
10 minutes after injection ..		212	40	165
3 hours after injection ..		222	68	235
27 hours after injection ..	52%	52	32	193

Rabbit No. 3. Injection of 9 cc. of distilled water intravenously.

	Hb	Barkan Iron Blood	Barkan Iron Serum	Heilmeyer Serum Iron	Bilirubin
		γ%	γ%	γ%	Mg. %
Before injection ..	65%	190	36	134	0.14
30 minutes after injection	59%	268	44	194	
5 hours after injection ..	47%	114	28	190	0.18
28 hours after injection	45%	98	10	121	0.22

of the haemoglobin and bilirubin in the course of the experimental haemolysis by intravenous injections of distilled water. The results are shown above.

These three examples show how informative the simultaneous determination of the fluctuations of the two iron fractions of *Barkan* and *Heilmeyer* can be when taken in the course of acute haemolysis. Actually phenylhydrazine provokes haemolysis which develops too slowly to be able to be followed systematically in animals.

As the changes in the non-haemoglobin iron often proceed rapidly, it is not surprising that *Barkan* failed to reach any conclusion as a result of his protracted observations. Moreover, in Observations Nos. 2 and 3 we can note that 27 and 28 hours after the acute haemolysis there was a reduction of the *Barkan* iron in the blood and serum, as this author had indicated in his experiments on chronic haemolysis. In the first observation, on the other hand, this decrease did not occur, doubtless owing to a second injection of distilled water which was given a few hours before the last sample was taken.

All these experiments show a certain analogy: The *Barkan* iron in the blood increases immediately after haemolysis, declining a few hours later to normal or even to definitely lower values. The *Barkan* iron of the serum shows on the whole inconsiderable fluctuations, with a tendency to increase between the first 30 minutes and 3 hours after the injection; after that it may fall to fairly low values. In general Heilmeyer's " serum iron " increases parallel with the degree of haemolysis and remains for from 3 to 5 hours at a higher level, after which it falls again, like the two other fractions of the non-haemoglobin iron.

If we compare the three sets of observations, the curves obtained show the following results with admirable unanimity:

After the first 10 minutes succeeding the intravenous water injection, the *Barkan* iron in the blood increases until it reaches a maximum between 10 and 30 minutes after haemolysis; 2 or 3 hours later these values again decline to normal; and 24 hours later they decline still further. Whereas *Barkan's* "easily split-off iron" rapidly increases in the blood after haemolysis, the two other serum fractions remain stationary and only rise after half an hour. From that time onwards the three fractions fall again to very low values, attaining the normal value 48 hours later.

We see therefore that there is a considerable difference in the course of the curves of the non-haemoglobin iron fractions which we have just analysed. This difference can also be noted in a series of observations which were conducted with the more complete

Haemolysis by addition of water

	Before		10 minutes after		3 hours after	
	Direct determination	Incineration of extracts	Direct determination	Incineration of extracts	Direct determination	Incineration of extracts
	γ%	γ%	γ%	γ%	γ%	γ%
Plasma:						
Water extract	0	180	0	130	0	85
6N HCl extract	69	165	97	140	83	160
Total incineration (plasma)		338		790 (330)*		868 (368)*
Erythrocytes:						
Water extract	2.5	1720	50	1500	40	1560
6N HCl extract	283		1160		1050	

*The values in brackets indicate the approximate quantities of iron contained in the plasma haemoglobin after haemolysis.

Mechanical Haemolysis

	Before		15 minutes after		5 hours after	
	Direct determination	Incineration of extracts	Direct determination	Incineration of extracts	Direct determination	Incineration of extracts
	γ%	γ%	γ%	γ%	γ%	γ%
Plasma:						
Water extract	2	32	5	145	3	204
6N HCl extract	56	395	105	360	148	390
Erythrocytes:						
6N HCl extract	458		478		588	

method of fractional determination of iron described above. We first endeavoured to follow *in vitro* the various fractions of the non-haemoglobin iron in haemolysis. In a first observation, by adding doubly-distilled water to the blood rendered uncoagulable with oxalate, we produced haemolysis. In order to avoid any possible dilution of the blood with the water we next, in a second observation, provoked the haemolysis by the mechanical destruction of a portion of the erythrocytes. The results obtained are as shown on page 83, and from these tables we obtain the values of the various iron fractions:

		Before	After 10 min.	After 3 hrs.
	Haemolysis by addition of water			
Erythrocytes	A-iron	3 γ%	50 γ%	40 γ%
	B-iron	780 γ%	1110 γ%	1050 γ%
	C-iron	934 γ%	290 γ%	470 γ%
Plasma	A-iron	0 γ%	0 γ%	0 γ%
	B-iron	69 γ%	97 γ%	83 γ%
	C-iron	110 γ%	43 γ%	77 γ%
	D-iron	159 γ%	320 γ%	340 γ%
	Mechanical Haemolysis			
Erythrocytes	A-iron	213 γ%	470 γ%	
	B-iron	245 γ%	8 γ%	
	C-iron	2329 γ%	3147 γ%	
Plasma	A-iron	2 γ%	5 γ%	3 γ%
	B-iron	54 γ%	100 γ%	145 γ%
	C-iron	339 γ%	260 γ%	242 γ%

The differences between the results of these two observations originate primarily from the fact that the mechanism of the haemolysis is not the same in both cases, and that the water needed to produce haemolysis in the first investigation had diluted the blood and hence also the percentage of the iron content. Thus these two observations are not directly comparable. Nevertheless, we are able to observe a certain agreement in the results of the fluctuations of certain fractions in the course of haemolysis. The loosely bound iron shows a marked increase in the plasma, also in some of the erythrocytes; the same applies to the firmly bound iron. On the other hand, the non-separable iron decreases. The iron of the protein precipitate shows rather a tendency to increase in the plasma.

Thus haemolysis liberates the loosely separable iron of the erythrocytes; but the passage of this iron to the plasma is a slow

process and is only partially effected. The iron which is bound to the great organic complexes is also released; the rapidity of its diffusion in the plasma is partly dependent upon the nature of the haemolysis. For instance, in mechanical haemolysis, in which the erythrocytes are not completely destroyed, this fraction appears rather to remain connected with the stroma of the erythrocytes. It can be assumed that haemolysis *in vitro* (as we were also able to observe *in vivo*) is followed by rapid mobilisation of the separable iron fractions (Iron A and B) from the destroyed blood cells, which, however, are only slowly diffused in the plasma.

Having followed *in vitro* the fluctuations of the non-haemoglobin iron in haemolysis, we next proceeded to a study *in vivo* of iron metabolism in acute haemoylsis. For this purpose we used dogs, large enough to furnish us with sufficient quantities of blood for the determination of the various fractions without this producing an excessive haemorrhagic anaemia. We proceeded as follows[1]:

The tables summarise the results of the two experiments:

Dog No. 1. 23 kg., 4 years old. Fasted for 15 hours. We removed 80 cc. of blood for analysis, then another 80 cc. which we defibrinated and haemolysed after diluting it with distilled water in the proportion of 1:3. Next, slow intravenous injection of 200 cc. of the haemolysed blood prepared in this way. The animal showed no resulting disturbance. After 1½ hours and again after 6 hours a fresh sample of blood was taken for analysis. The autopsy which followed showed absolutely healthy organs. It was remarkable to note that the intervention had not caused any kind of oedema.

Dog No. 1

	Before		1½ hours later		6 hours later	
	Direct determination	Incineration of extracts	Direct determination	Incineration of extracts	Direct determination	Incineration of extracts
	γ%	γ%	γ%	γ%	γ%	γ%
Erythrocytes:						
Water extract	206	370	268	350	328	440
6N HCl extract	233	501	268	330	318	410
Serum:						
Water extract	28	37	74	125	10	77
6N HCl extract	123	330	288	330	35	185
Total ash reduction		400		572*		220*

*These figures represent the value of the total incineration minus the value of the calculated haemoglobin iron found in the serum after haemolysis, according to Hb determination.

[1] We were able to conduct these experiments through the kind co-operation of the Physiological Institute of the University of Lausanne, and we wish to take this opportunity of thanking Professor A. Fleisch for his great kindness and valuable advice.

Dog No. 2. 14 kg., 3 years old. Fasted for 15 hours. 60 cc. of blood withdrawn for the purpose of analysis; next, intravenous injection of 85 cc. of distilled water rapidly in amounts of 2–4 cc. at a time. The animal showed slight transient disturbance in the respiratory rhythm. After 2, 7 and 9½ hours blood was withdrawn for analysis. As the second blood taken showed comparatively slight haemolysis another injection was given 4 hours after the start of the experiment, with 25 cc. of distilled water in one intravenous injection. After 5 seconds the dog showed transient temporary disturbances of respiration. After 1 hour the pulse rose, the breathing became irregular and oedema gradually set in. We interrupted the experiment in order to avoid further errors in determination due to the disturbance in the distribution of water.

Dog No. 2

	Before	After 2 hours	After 7 hours	After 9½ hours
Serum:	γ%	γ%	γ%	γ%
Water extract	10	72	8	8
1.2% HCl extract	72	95	20	12
6N HCl extract	150	210	40	50

The tables permit us to calculate the following values of the various iron fractions:

		Before	After 1½ hrs	After 6 hrs
		γ%	γ%	γ%
Erythrocytes	A-iron ..	206	268	328
	B-iron ..	27	0	0
	C-iron ..	268	82	112
Serum	A-iron ..	28	74	10
	B-iron ..	95	214	25
	C-iron ..	207	42	150
	D-iron ..	70	242	35
Total iron		400	572	220

We can therefore draw the inference that, in our experiments on haemolysis in dogs, one and a half hours after haemolysis *in vivo* there occurred an increase of the circulating A-iron in the erythrocytes and serum, with a reduction of the B-iron in the erythrocytes. At the same time there was marked increase of this fraction in the serum, diminution of the non-separable iron in the erythrocytes and serum, and conspicuous increase of the iron of the protein precipitate in the serum. On the other hand, as early as six hours after haemolysis the serum showed a reduction in most of the serum fractions, with the exception of the non-separable iron, which possibly cannot be directly utilised for blood regeneration, if proceeding from the bone-marrow. In the erythrocytes liberation of the A-iron proceeded slowly; but the B-iron (strongly bound iron), which passed rapidly into the serum, appeared still to be absent from the red cells. From this it can be concluded that, in

the dog, haemolysis is characterised by a mobilisation of the separable iron of the erythrocytes, which passes quickly into the plasma. The non-separable iron decreases considerably in both blood and serum, either because it is fixed by the reticulum which co-operates closely in the mechanism of blood decomposition, or because it associates itself with the serum proteins which correspond to the iron of the protein precipitate. But during the blood regeneration in the bone-marrow, which follows as early as six hours after haemolysis, we see a distinct reduction of the two separable iron fractions; this appears to indicate that the organism makes use chiefly of these two fractions (A and B) for the work of blood regeneration. In contrast to this, the iron of the large organic, non-separable molecules increases slightly after the phase of acute haemolysis, and the iron which is bound to the serum protein shows a tendency to return to normal values.

As in haemolysis *in vitro*, we also found *in vivo* a preliminary liberation of the easily split-off iron of the red cells, which becomes partly distributed in the plasma. The two fractions A and B (iron easy and hard to split off) diminish considerably as early as six hours after haemolysis; they are doubtless needed for other functions, the most important of which is haematopoiesis. The iron bound up with the protein complexes of the plasma shows a temporary rise immediately after haemolysis. This finding appears to show that the intravenous injection of water, together with the accumulation of liberated haemoglobin in the plasma, produces a temporary change of the physico-chemical balance of the blood proteins.

These considerations therefore permit us to assume that there actually exist in the circulating blood various iron fractions in the form both of easily-separable complexes and of not-easily-separable and non-separable complexes; each of these behaves differently in the course of haemolysis. In this way are to be explained the fluctuations of iron which have been described and which were observed during haemolysis, according to the methods of *Barkan* and of *Heilmeyer*. It is, above all, the iron of the separable complexes and the iron of the erythrocytes extracted by HCl that suffers the greatest fluctuations in the course of haemolysis and which is most quickly transferred from the erythrocytes to the plasma. The iron that is irreversibly bound to the complexes is less influenced by haemolysis and shows only a slight tendency to stray from the blood corpuscles into the blood plasma.

All these reflections, of course, in no wise affect *Barkan's* hypothesis regarding the presence in the circulating blood of pseudohaemoglobin; the latter, which represents an intermediary stage between haemoglobin and bilirubin, should afford us par-

ticular interest in connection with the study of haemolysis. As a matter of fact, it is quite possible that the iron fraction extracted by HCl corresponds, in part at least, to Barkan's pseudohaemoglobin.

Having now described these experimental findings we will next proceed to a study of certain clinical data connected with the iron metabolism occurring during haemolysis. We begin with some cases which were treated with phenylhydrazine.

C. C., aged 35 years. (See Diagram 4.)

	Hb	E	L	Barkan Iron Blood	Barkan Iron Serum	Heilmeyer Serum Iron	Bilirubin
Feb. 23	83%	4.7 Mill.	3,200	960 γ%	112 γ%	125 γ%	1.06 mg.%
Feb. 24	The patient receives 0.1 g. phenylhydrazine three times daily.						
Feb. 26	80%	4.7 Mill.	4,100	500 γ%	80 γ%	80 γ%	1.22 mg.%
	The treatment is continued with 0.2 g. phenylhydrazine daily.						
Feb. 28	78%	4.54 Mill.	3,900	2,340 γ%	134 γ%	209 γ%	1.40 mg.%
Mar. 1	73%	4.51 Mill.	5,400	1,660 γ%	256 γ%	297 γ%	1.67 mg.%
Mar. 4	End of treatment with phenylhydrazine.						
Mar. 6	71%	4.27 Mill.	5,300	390 γ%	178 γ%		1.57 mg.%
Mar. 8	67%	4.02 Mill.	4,100	280 γ%	112 γ%	165 γ%	1.50 mg.%
	The blood picture now shows signs of increased blood regeneration (anisocytosis with predominance of macrocytes, increase of reticulocytes, etc.).						
Mar. 13	71%	3.89 Mill.	3,800		36 γ%	85 γ%	1.50 mg.%
Mar. 15	69%	3.53 Mill.	3,900	630 γ%	79 γ%	120 γ%	1.45 mg.%

B. E., aged 69. Polycythaemia.

	Hb	E	L	Barkan Serum Iron	Heilmeyer Serum Iron	Bilirubin
Oct. 3	114%	8.42 Mill.	26,800	60 γ%	67 γ%	0.85 mg.%
Oct. 4	Phenylhydrazine treatment for 10 days.					
Oct. 9	107%	8.26 Mill.	27,400		225 γ%	1.13 mg.%
	After end of treatment:					
Oct. 24	105%	7.89 Mill.	26,200	66 γ%	96 γ%	1.15 mg.%
Nov. 14	92%	7.48 Mill.	26,500	28 γ%	96 γ%	
	Second treatment with phenylhydrazine:					
Nov. 28	109%	6.15 Mill.	29,900	32 γ%	90 γ%	1.08 mg.%
	Phenylhydrazine treatment:					
Dec. 2	107%	6.06 Mill.	27,100	288 γ%	329 γ%	11.11 mg.%
	After the treatment:					
Dec. 21	92%	6.16 Mill.	34,700	112 γ%	180 γ%	0.96 mg.%
	Two months later:					
	94%	5.48 Mill.		73 γ%	95 γ%	0.98 mg.%

B. F., aged 45.

	Hb	E	Bilirubin	A-iron	B-iron	C-iron	D-iron	Total iron
May 23	98%	6.9 Mill.	0.35 mg.%	25 γ%	123 γ%	82 γ%	40 γ%	270 γ%
	Rep. Phenylhydrazine, 1.0 g. in 10 days.							
May 28	97%	6.1 Mill.		60 γ%	130 γ%	40 γ%	60 γ%	290 γ%
June 2	87%	5.3 Mill.	0.51 mg.%	76 γ%	119 γ%	105 γ%	74 γ%	374 γ%

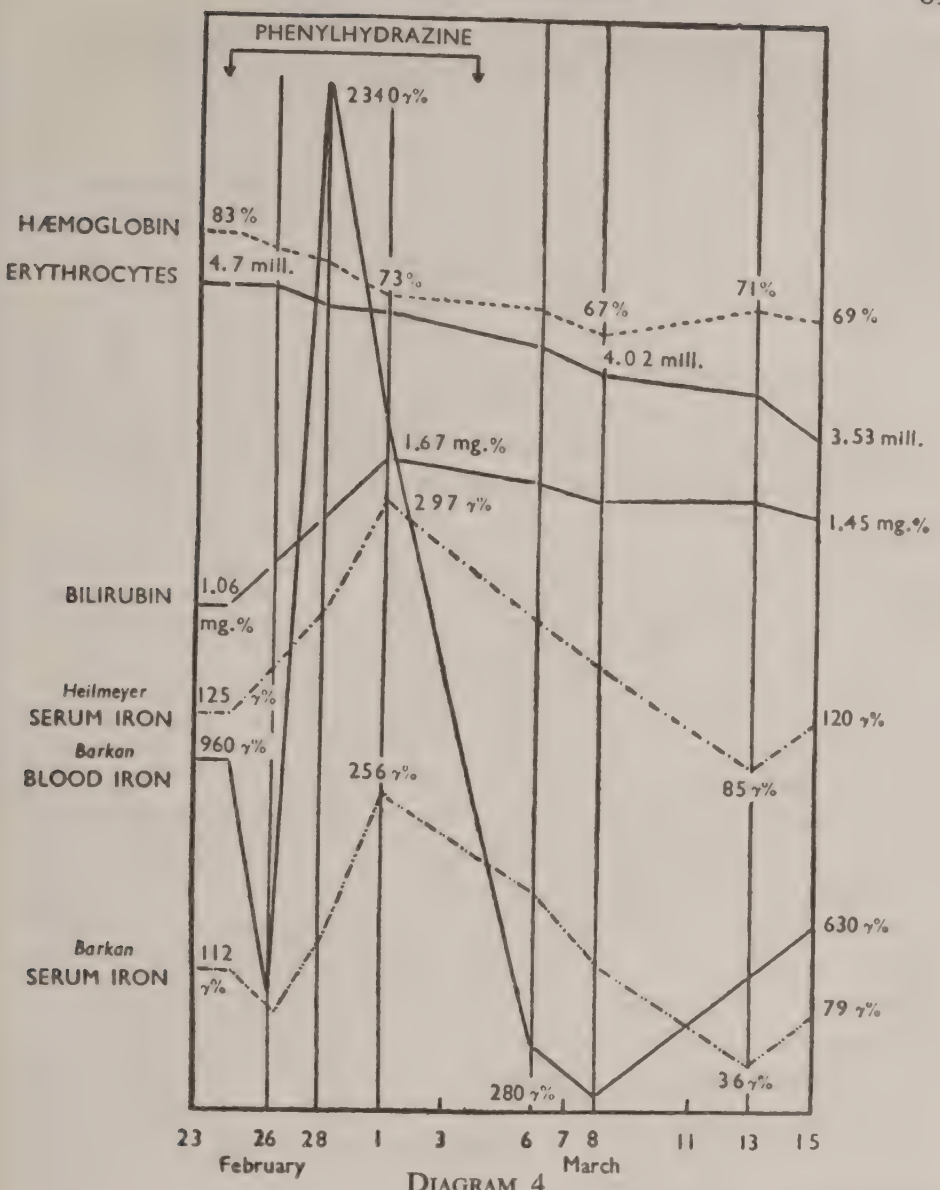

DIAGRAM 4
Iron metabolism in a case of haemolysis.

In these few clinical examples the haemolysis is characterised by a liberation of iron from the time that the destruction of the blood corpuscles begins. We find this iron again in the serum, above all in an easily-separable form. In this way the *Barkan* fraction receives in the course of haemolysis a comparatively greater increase than does the *Heilmeyer* iron fraction. The determination of the serum iron fractions, according to our own technique, clearly shows the following phenomenon: In the relatively slight haemolysis with slow evolution of Case B. F., page 88, the easily-separable iron of the serum (Iron A) is trebled, whilst the values of the other fractions increase very much less, both relatively and absolutely.

In pernicious anaemia we also often find, as a symptom of marked haemolysis, comparatively high values for the easily-separable iron.

Examples	Hb.	Erythrocytes	Barkan Serum Iron	Heilmeyer Serum Iron
Mrs. L. J., aged 40	52%	1.78 Mill.	110 γ%	181 γ%
Mr. A. H. „ 60	55%	2.25 Mill.	118 γ%	201 γ%

The determination of the serum iron according to our four fractions serves in pernicious anaemia to emphasise more clearly this predominance of the separable iron fraction, and particularly that of the easily-separable, as compared with the non-separable, iron.

	Hb.	Erythro-cytes	A-iron	B-iron	C-iron	D-iron
1. R. B., aged 46	46%	1.26 Mill.	115 γ%	245 γ%	45 γ%	25 γ%
2. T. J., „ 60	57%	2.0 Mill.	75 γ%	145 γ%	70 γ%	30 γ%

As a result of the action of liver extracts the fractions A and B rapidly decline, especially that of the easily-separable iron. The liver treatment has the effect of creating conditions which to a certain extent oppose haemolysis; but bone-marrow regeneration here still continues to exert a strong influence on the iron metabolism, and we shall consider these patients again later on when studying protein metabolism in connection with erythropoiesis.

It seems of value to reproduce at this point the values of *Barkan* in the total blood, as determined by *Vannotti* in 1937 in a case of paroxysmal cold-haemoglobinuria. With their help it is possible to follow the fluctuations of the iron before, during and after the attack of haemoglobinuria.

	Hb.	Barkan iron in blood
3 days before the attack ..	70%	950 γ%
„ „ „ „ „ ..	68%	750 γ%
1 day „ „ „ ..	65%	980 γ%
Immediately before attack ..	69%	1120 γ%
At onset of attack		1850 γ%
Towards end of attack ..	58%	2530 γ%
2 hours after end of attack ..		2100 γ%
6 „ „ „ „ „ ..	46%	900 γ%
12 „ „ „ „ „ ..	48%	490 γ%
24 „ „ „ „ „ ..	54%	515 γ%
48 „ „ „ „ „ ..	58%	880 γ%

Before concluding this section we wish to give the results of

a few experiments we made in connection with haemoglobin decomposition in bone-marrow cultures *in vitro*. In these experiments (*Vannotti* and *Siegrist*), which will be treated at length in the next section, haemoglobin was brought into contact with small quantities of liver, with sterile precautions, resulting in rapid destruction and conversion of the blood pigment. The speedy diminution of the haemoglobin in the culture was soon followed by the formation of considerable quantities of bilirubin, accompanied by a decided increase of the two iron fractions (according to *Barkan* and *Heilmeyer*). In these observations the *Barkan* iron also showed a tendency during haemoglobin decomposition to increase more rapidly than the *Heilmeyer* serum iron. From this we are able to conclude that the change in the various iron fractions in the course of haemolysis is roughly similar in human and animal experimentation to what it is in our experiments *in vitro*. Acute haemolysis mobilises separable non-haemoglobin iron in the circulating blood; this iron is present in the erythrocytes either as respiration iron of the erythrocytes or as active transport iron; finally it may be the intermediary product of the physiological decomposition of haemoglobin. It is also possible that acute haemolysis may expedite the process of this extra-hepatic haemoglobin decomposition, thus provoking a liberation of the iron from the molecule of the blood pigment. A more fundamental conversion of these products of haemolysis by the various organs of pigment metabolism (reticulum, spleen, liver) leads, during a later phase of this process, to the appearance of other final products of this haemoglobin decomposition, i.e. bilirubin and iron. Thus it happens that in chronic haemolysis the processes described as occurring in acute haemolysis are not all repeated in detail. In such cases there is usually an increase of *Heilmeyer's* serum iron, which may last for several days, whereas the increase of *Barkan* iron is usually of a more temporary nature. It should be stated, however, that an increase of serum iron does not always indicate haemolysis. It may be of varying clinical significance, as we shall later see. The condition can only be considered haemolysis if the increased serum iron content is accompanied by an addition of definite iron fractions and of bilirubin or by increased urobilin elimination.

B. *Disturbances of Erythropoiesis*

Before entering in greater detail into the question of iron metabolism during erythropoietic disturbances we wish briefly to recall certain data relating to normal iron metabolism.

Recently absorbed iron appears in the blood, where it forms part of the non-haemoglobin iron. Its chief function is to provide

the organs of erythropoiesis with raw material for the synthesis of haemoglobin, and the tissues in general for the metabolism of the cell ferments. In a second capacity it is able to complete the iron reserves which are available for the organism in the liver, spleen, bone-marrow, etc. Finally, but only once the more pressing needs of the tissues have been satisfied and the reserves of the organism have been replenished, the freshly absorbed iron serves to maintain the non-haemoglobin iron content of the serum at a constant level. This value is usually higher in men than in women, probably owing to the chronic haemoglobin losses suffered during menstruation. This content varies but little under physiological conditions; it hardly even varies as a result of meals, although certain changes may occur in the different fractions. If there is no anaemic condition that needs to be restored and no reserves have to be supplemented the organism will need but a slight amount of iron, in which case absorption will sink to practically nil, in accordance with this condition. Under pathological conditions, on the other hand, if there is iron deficiency following upon haemorrhage, or as a result of a chronic disturbance of iron absorption through the intestine, the content of non-haemoglobin iron in the blood will fall, as soon as the iron needs of the tissues exceed the quantities supplied by the food and the reserves of the deposit organs. The non-haemoglobin iron of the serum, which only attains its normal value once all the iron needs of the organism have been satisfied, thus offers a reliable picture of the iron needs of the organism and of the condition of its reserves. If, however, the body disposes of more iron than it can utilise at the time, the blood iron content will temporarily exceed the usual normal values. As we have seen, this is the case in haemolysis.

Thus the serum iron content gives us important aetiological data and, as we shall see, it can offer valuable indications for the treatment of conditions of iron deficiency, as for instance in the various forms of anaemia.

A systematic study of iron metabolism in anaemia has shown us that there exist some clinical forms characterised by an invariably low, others by a normal, and still others by an increased non-haemoglobin iron content. On the other hand, it is well known that certain forms of anaemia fail to react to therapeutic administration of iron. Hence we must assume that certain types of anaemia are not directly associated with disturbances of iron metabolism. Blood pigment consists of three factors: iron, chromogen (which chemically is a porphyrin) and globin. It would therefore appear that the occurrence of anaemia does not necessarily indicate iron deficiency; doubtless the other two constituents

of blood pigment can also produce such a condition. In addition, we are acquainted with forms of anaemia which originate from a certain inertia in the functioning of the bone-marrow, and still others from a certain dysfunctioning of the bone-marrow, in which condition haemoglobin formation is not necessarily involved to the same extent as in the formation of erythrocytes.

If, then, we wish to undertake a systematic study of iron metabolism in relation to the various forms of anaemia, it is advisable, in the interests of greater clarity, to divide the latter into two broad categories: on the one hand, the anaemias caused by iron deficiency, corresponding to *Heilmeyer's* admirable concept (that is, the anaemias with lowered serum iron content); and on the other hand, the anaemias which show no lowered serum iron content and in the pathogenesis of which iron deficiency plays only a secondary, subordinate part, or even no part at all. This sub-division naturally does not apply to all conditions of blood deficiency; it merely serves to help us set up the following classification:

1. *The Iron-Deficiency Anaemias*

An abnormally low serum iron content characterises a large group of anaemias which, in addition, have quite a number of symptoms in common, varying in intensity according to the individual cases, but all of which derive from the same cause, i.e. iron deficiency. These forms of anaemia are as a rule extremely hypochromic, which is easy to understand, since the iron represents 3.35% of the weight of the haemoglobin, whilst in cell formation it is in only a 20–100-times smaller concentration. Hence the iron deficiency will above all affect the synthesis of the haemoglobin, and the formation of the red blood cells may therefore often be totally unaffected. In addition to this, iron performs various different functions in the organism—as material for the building up of cells and as a catalyst—so that, for example, a lack of iron would in the long run affect other important functions. It is thus that *Heilmeyer* and *Vannotti* explain adynamia, which in certain hypochromic anaemias is very marked and which frequently subsides long before the erythropoiesis has become normal. Finally the pathological friability of the finger nails and the painful rhagades at the corners of the mouth, which such patients often show, appear also to be clinical manifestations of the same deficiency, inasmuch as the renovation of the especially exposed layers of skin, consequent upon a lack of the factors of growth, does not occur with sufficient rapidity.

The causes of iron deficiency are manifold; here we shall only describe the chief ones, with the help of clinical examples.

The first example is given by acute anaemia after haemorrhage. In acute haemorrhagic anaemia *Barkan, Heilmeyer, Thoenes* and *Aschaffenburg, Vannotti, Walker Burnham* and *Fitz* and others observed a decided diminution of the non-haemoglobin iron. The best explanation of this decrease is, in our opinion, that of *Heilmeyer*. Iron decrease after a heavy haemorrhage results from: (1) a direct loss of iron as a result of bleeding; (2) a dilution of the blood mass by the compensatory addition of tissue fluid; (3) increased iron consumption by the bone-marrow for the purpose of balancing the blood regeneration.

The last-named aetiological possibility, which was previously mentioned by *Fontès* and *Thivolle* and supported by *Vannotti*, is intimately connected with the problem of the involvement of the non-haemoglobin iron in haemoglobin formation—a problem to which we wish to give our particular attention in this section. We are able to prove this hypothesis with the aid of a number of clinical observations; but we prefer first to describe a group of experiments (*Vannotti* and *Siegrist*) which were undertaken for the purpose of studying in greater detail the problem of the function of the bone-marrow.

It was planned to follow by experimental methods the fluctuations of the haemoglobin content, of *Heilmeyer's* serum iron and of *Barkan's* easily split-off iron in bone-marrow cultures under varying conditions. These experiments represent a contribution to the study of haemoglobin formation in the bone-marrow. At first, haemoglobin is found in the erythroblasts in very small quantities, which increase as the cell advances to maturity, whilst the basophil protoplasm gradually becomes acidophil. The blood pigment is probably formed in the nucleus of the erythroblast, which is supplied with iron. But chemically considered, this does not yet solve the problem of haemoglobin synthesis. What are the substance and the intermediary products on which haemoglobin formation is based? From the investigations conducted by *H. Fischer* and his school we know that blood pigment is formed by a porphyrin ring, consisting of four pyrrole nuclei, which contains iron in its centre. Hence the chemical structure of haemoglobin shows (see formulae on pages 43 and 52) close connections with the porphyrins (ring with four pyrrole nuclei without iron). The investigation of haemoglobin synthesis also includes that of iron metabolism and of the pigments which are so closely related to blood pigment. The porphyrins are regularly found in the bone-marrow where, according to *Borst* and *Königsdörfer*, they represent the pre-forms of haemoglobin during the first period of embryonic life. As regards bilirubin, it is a regular product of blood pigment decomposition; in the opinion of

various authors it might in part be utilised by the organism for haemoglobin synthesis by the bone-marrow.

Regarding the influence of other organs, so closely bound up with erythropoiesis as to render it almost impossible to examine this problem *in vivo*, *Vannotti* and *Siegrist*, and later *Blum*, analysed the conversion of these pigments and of iron in isolated bone-marrow cultures. These bone-marrow cultures were prepared according to the directions of *Osgood* and *Brownlee* and the quantitative determination of the pigments according to the spectrophotometric method (for details, see the publication of *Vannotti* and *Siegrist*).

If small quantities of haemoglobin in an isotonic solution are added to a bone-marrow culture, it is possible to observe with fair regularity a slightly progressive increase of the haemoglobin content. But this soon ceases and after ten to twenty hours it will have fallen to its initial value. The explanation of this phenomenon is offered by the determination of the non-haemoglobin iron and of the bilirubin in the nutrient medium. Actually, to the extent that the haemoglobin content increases, the iron content diminishes; but on the other hand, as soon as the haemoglobin curve begins to sink the iron increases until it considerably exceeds its initial value. At the same time the bilirubin content increases.

Hence the bone-marrow is able to synthesise small quantities of blood pigment in the cultures; for this it utilises their iron reserves until such time as the accumulated products of decomposition render living conditions within the culture too unfavourable. Autolysis then sets in, accompanied by haemoglobin destruction (the *Hüfner* quotient in the spectro-photometric determination begins to change), and this is followed by the appearance of bilirubin and non-haemoglobin iron. This decomposition of the blood pigment is probably due to the reticulo-endothelial system which it was not possible to remove from the cultures. If small quantities of liver parenchyma are added to the nutrient medium, the destruction of the haemoglobin is effected in a much more complete and rapid manner and is accompanied by a release of iron and the formation of vast quantities of bilirubin. After the addition of serum to a bone-marrow culture, there is more conspicuous haemoglobin formation and this is sometimes proportional to the added amounts of serum. As regards the iron concentration of the cultures, this diminishes considerably as haemoglobin increases. But the determination of the quantitative non-haemoglobin iron shows us that the fluctuations of the blood pigment and iron proceeding in contrary directions are not absolutely inversely proportional to one another (see Diagram 5).

We think it not without interest to determine at this point the

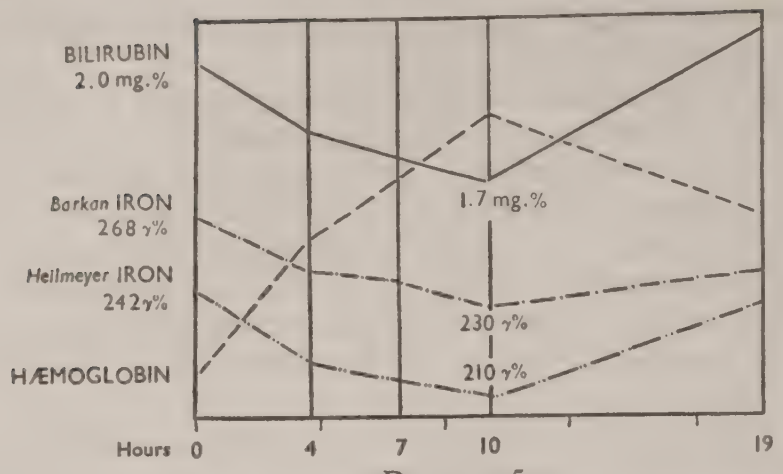

DIAGRAM 5
Changes in blood pigment and iron in bone marrow culture after addition of serum.

decline of *Barkan's* easily split-off iron during haemoglobin synthesis. This determination, which was often repeated, has led us to assume that even *Barkan's* pseudohaemoglobin, i.e. the intermediary product of haemoglobin decomposition to bilirubin, may, judging from our observations *in vitro*, take part in the re-synthesis of the blood pigment. In our experiments the pseudohaemoglobin was derived from the erythrocytes of the bone-marrow, from the serum and probably from reticular activity.

The intensity of the haemoglobin synthesis in the bone-marrow depends on many factors: firstly, upon the body's need of haemoglobin and, secondly, upon the quantity of substances available for pigment formation; finally, it is also associated with the functional capacity of the bone-marrow. The activity of blood formation is further intimately connected with the general needs of the organism; the marked reaction of the bone-marrow following upon every case of acute anaemia; the reticulocyte crisis in pernicious anaemia as a result of liver treatment; the reaction of the bone-marrow in high altitudes, etc.; all these offer sufficient proof of this.

The activity of the bone-marrow furthermore depends on the supply of the necessary synthetic substances, as is clearly seen from our experiments. There can be noted an increase of haemoglobin production after the addition to the culture of serum, as also of amino acids in the form of tryptophane and histidine (*Blum*). Serum which contains bilirubin, pseudohaemoglobin and iron in sufficient quantities is shown to be a highly favourable medium for blood pigment formation, and finally tryptophane and histidine, which contain the pyrrole nucleus, might also partake in the formation of the porphyrin ring.

Finally, if a great quantity of bilirubin is added to the serum

(for example, the serum of an icterus patient) the haemoglobin formation will not exceed the values of production which are attained with normal serum. The increase of one single constituent part of the serum does not therefore suffice to increase haemoglobin production: a fact that illustrates the co-operation existing betweeen the various elements of haemoglobin synthesis in the building up of the blood pigment. These considerations serve to explain the observation of *Vosskühler* to the effect that the bone-marrow does not take up more iron than it needs—an observation which we were able to confirm by comparative analyses of sternal blood and ordinary blood in cases of anaemia showing both increased and lowered iron content.

The observations of *Seggel*, *Vannotti*, and recently of *Wintrobe* and his collaborators, show that in cases of great erythropoietic reaction characterised by a high reticulocytosis and by the formation of haemoglobin, as may be seen in anaemias due to acute blood loss, there is an important increase of protoporphyrin in the blood at the time of the reticulocyte crisis. This fact made *Seggel* think that reticulocytes were erythrocytes containing protoporphyrin (fluoroscytes). At the moment when the percentage of non-haemoglobin iron is the lowest and the protoporphyrinaemia is the highest, we must admit with *Wintrobe* and his school that, owing to the temporary lack of iron and an accelerated synthesis of haemoglobin, a blood pigment without iron is formed: protoporphyrin. This pronounced protoporphyrinaemia is not only found in acute haemorrhage, but also in all pathological process provoking an increase of haemoglobin production. This is the case in pernicious anaemia at the time of the reticulocyte crisis, also in the anaemia which develops in the course of an acute or subacute infectious disease.

Wintrobe and his collaborators (*Cartwright*, *Greenberg* and others) studied carefully the problem of the infectious anaemia. These authors found that the non-haemoglobin iron curves after oral administration of iron are flat. This fact can be compared with the observations of *Hahn*, *Bale* and *Whipple* concerning a decrease of iron absorption by the intestine during the infection.

Moreover, *Wintrobe* and his collaborators showed that intravenously injected iron disappears rapidly from the circulation. However, this iron does not go into the bone-marrow in order to provoke an increase of haemoglobin, but it is deposited in greater quantity in the liver and spleen, i.e. in the storage organs. These authors observed, furthermore, that tissues subjected to an inflammation do not grow richer in iron. The injected radioactive iron does not deposit itself in an elective manner. In their recent observations, the American authors could note that, with the help

of continued intravenous infusions of iron, the percentage of iron does not increase in the plasma; it remains low and is not accompanied by an increase of haemoglobin. Thus, these authors arrive at the conclusion that if one were to suppose that the anaemia of infection is due to a failure to form "haem" as the result of a lack of iron, this hypothesis could not be maintained and should be replaced by the hypothesis of a failure to utilise iron by the synthesis of the haemoglobin. This fact could explain the rapid passage of iron into the storage organs and could be related to a disturbance of the metabolism of proteins which are necessary for the synthesis of the haemoglobin.

Moreover, the very low percentage of non-haemoglobin iron must, in our opinion, find its principal explanation in the disturbances of the metabolism of proteins during the inflammation. These disturbances are often characterised by a quantitive and qualitative modification of the distribution of plasma proteins, which are carriers of iron, and also by a modification of the iso-electric point of these proteins, a modification which can provoke a decrease, often important, in the links that fix iron to its support. The iron which thus is not joined to the proteins is deposited rapidly in the storage organs, without being able to participate in haemoglobin synthesis (*Neukomm*).

It is not excluded that the anaemia associated with cancer may also have analogous origins and cause, with its low percentage of non-haemoglobin iron, a decrease in the formation of haemoglobin and even a tissue anaemia with a decrease in cytochrome C (*Vannotti, Prader* and *Gobat*).

(*a*) *Anaemias due to haemorrhagia.*

On page 94, we quoted *Heilmeyer's* opinion in order to explain the reasons for the post-haemorrhagical hyposideraemia. This author was able to make the following observations:

In normal individuals a venesection of 500–800 cc. rapidly (as soon as six hours later) brings about a distinct lowering of the non-haemoglobin iron in the serum. In comparison this is much more than that of the haemoglobin and erythrocytes. Thus, while the erythrocyte and haemoglobin reductions were 13% in one case and 17% in another, the reduction of serum iron in the same cases was 20% and 30%, respectively. The considerable decrease of serum iron cannot therefore be attributed exclusively to loss and dilution of blood, in which case the same relative effect should apply to the number of red blood cells and the haemoglobin concentration. *Heilmeyer* sees in this a proof of the fact that a certain proportion of the circulating iron from the bone-marrow is used for blood regeneration.

On the other hand, the iron curve shows a rapid change twenty-four to twenty-eight hours after the venesection. After the preliminary decrease there is a simultaneous rise of both the serum iron and the number of reticulocytes. *Heilmeyer* attributes this great increase of iron, which is unconnected with haemolysis, to the mobilisation of the deposit iron of the organism for the purpose of maintaining the effort of blood regeneration by the bone-marrow.

On various occasions we were able to repeat this observation of *Heilmeyer* of a temporary increase of iron on the day after a venesection of from 250–300 cc. Here are two examples:

1st case:	Before venesection	Serum iron 130 γ%
	24 hours after venesection..	„ „ 220 γ%
2nd case:	Before venesection	Serum iron 98 γ%
	24 hours after venesection..	„ „ 195 γ%

Heilmeyer is correct in recognising a distinct difference between the state of the iron following venesection and that after a regular haemorrhage. Twenty-four hours after a severe haemorrhage this author was never able to note any increase of the serum iron. He is of the opinion that this difference in the reaction is primarily due to the fact that in venesection the loss of blood is relatively small ($\frac{1}{10}$ of the total quantity of blood), whilst in the haemorrhages which he was able to follow the blood losses were considerable, some cases even exceeding half the total quantity of blood. In such extreme cases the organism loses enormous amounts of iron circulating in the blood and at the same time exhausts the iron reserves, which it naturally can no longer mobilise after twenty-four hours.

As a matter of fact, in cases of severe haemorrhage a continuous diminution of the serum iron can be noted. In slight haemorrhages a temporary increase can sometimes be seen after twenty-four hours. In our opinion this increase of iron does not only depend upon the amount of blood lost, but upon a number of clinical factors, particular to each individual case. Here are a few examples:

Case 1: Woman, aged 29. General condition good. Heavy gastric haemorrhage resulting from a gastric ulcer. For several hours vomiting of great quantities of blood. Comparatively slight melaena.

1 month before haemorrhage	Hb. 98%		
6 hours after haemorrhage ..	Hb. 55%	serum iron	62 γ%
28 hours after haemorrhage ..	Hb. 38%	„ „	131 γ%
3 days later	Hb. 40%	„ „	58 γ%
1 month later	Hb. 87%	„ „	120 γ%

The general condition of the patient was not greatly affected by this severe haemorrhage and the bone-marrow reacted promptly. In this case (the only haemorrhage) the iron level was about the same as after venesection, as the organism was not weakened at the time of the loss of blood.

Case 2: Man, aged 26. Pulmonary tuberculosis with cavities. General condition poor, with excessive weakness and emaciation; slight pre-existing anaemia; Hb. 65%. Marked haemoptysis.

24 hours after	Hb. 59%	serum iron 32 γ%
3 days later	Hb. 58%	„ „ 30 γ%
1 month later	Hb. 62%	„ „ 38 γ%

In this case the reaction of iron mobilisation is absent, although the quantity of blood lost by haemoptysis was comparatively little. But we are here confronted by an individual whose general reaction was bad, the bone-marrow never having shown more than a slight tendency to blood regeneration and whose content of circulating iron was very low. The mobilisation of the iron reserves after the loss of blood was absent, due to the two following reasons: (a) absence of the reaction of mobilisation by the bone marrow; (b) deficiency of deposit iron.

Case 3: Woman, aged 53. Cirrhosis of the liver, gastro-intestinal haemorrhage due to rupture of an oesophageal varicose vein. One single haematemisis, but pronounced melaena for one and a half days. Even previously the general condition was poor. The patient was greatly affected by the haemorrhage. The secondary anaemia which followed was very long drawn-out, although after the haemorrhage there was a certain tendency to regeneration.

		Barkan iron	Heilmeyer serum iron
24 hours after haemorrhage	Hb. 40%	83 γ%	95 γ%
3 days after „	Hb. 52%		138 γ%
1 week after „	Hb. 50%	102 γ%	100 γ%
1 month later	Hb. 42%	75 γ%	100 γ%

The serum iron ceased to increase after twenty-four hours, but showed a tendency to rise for a certain length of time (three days) after the haemorrhage. This rise, which was not followed by perceptible regeneration of the bone-marrow and which also was not due to haemolysis, resulted in our opinion from partial resorption of the iron through the intestinal wall, due to the presence of considerable quantities of blood in the digestive tract.

The chromogen also takes part in the regeneration of blood pigment after loss of blood. In this way is to be explained the mobilisation of iron-free haemoglobin, i.e. porphyrin, at the time when the serum iron diminishes after a haemorrhage. The increase of porphyrin, frequently observed (*Vannotti*), and which corresponds to the increase of the fluorescytes in experimental acute anaemia, was studied in detail by *Langen and Grotepass*, who found that porphyrin formation stops as soon as erythropoiesis is exhausted by a fresh haemorrhage.

After these few remarks on the value of non-haemoglobin iron under the influence of severe and acute losses of blood (venesection, acute haemorrhages), we wish to bring up the subject of the fluctuations of the non-haemoglobin iron in the course of slight, concealed chronic haemorrhages which almost invariably involve the slow evolution of more or less severe forms of secondary anaemia. In such cases we usually find a reduction of the non-

haemoglobin iron (*Heilmeyer* and *Plötner, Loeke, Main* and *Rosbach, Vannotti,* etc.). *Heilmeyer* and *Plötner* wondered whether this reduction was due, as in acute cases of loss of blood. to a greater consumption of iron in connection with blood regeneration. These authors insisted above all on the fact that the reduction of the iron is in general much more pronounced than that of haemoglobin. Hence in this case also the possibility must be envisaged that blood dilution might be the cause of the reduction of iron values. The symptoms of blood-forming regeneration were either entirely absent or insignificant. In the anaemias caused by concealed chronic haemorrhage only a slight increase of the reticulocytes can be noted; sternal puncture yields no evidence of great activity of the bone-marrow. Finally, the organs involved in haemoglobin metabolism do not in such cases show great quantities of reserve iron (*Whipple, Hahn,* etc.). Hence the reduction of the serum iron is not only due to the claims of the blood-forming organs in connection with pigment formation; *Heilmeyer* holds that it might even be attributed to an impediment in the process of blood destruction, i.e. to diminished haemolysis. In their publications on urobilin balance and the elimination of haemoglobin derivatives *Heilmeyer* and *Anton* have rejected this possibility and have accordingly reached the conclusion that the reduction of circulating non-haemoglobin iron in chronic cases of haemorrhagic anaemia is due to direct loss of iron as a result of repeated and protracted haemorrhages, which finally result in an impoverishment of the iron reserves of the organism.

Heilmeyer's hypothesis is confirmed by the observations of *Whipple* and his collaborators, who showed in dogs that after repeated venesection the iron deposits in the liver, spleen and other organs were greatly reduced. Moreover, the iron balances indicate that individuals suffering from anaemia and showing a very low content of circulating iron will, as a result of iron administration, soon begin to retain large quantities of iron which, therefore, contrast with the conditions prevailing in the normal organism. This abnormal iron retention serves to conceal the iron deficiency caused by the chronic haemorrhage. It must, therefore, be assumed that the conspicuous reduction of serum iron in chronic haemorrhagic anaemia results from a great loss of iron during the chronic haemorrhage.

However, it should be remarked that during the simultaneous determination of *Heilmeyer's* serum iron and of *Barkan's* easily split-off iron during chronic haemorrhagic anaemia we were always able to determine not only a very low content of *Heilmeyer's* serum iron, but also a relatively smaller fraction of *Barkan's* iron (in some cases only traces, sometimes not even any at all).

	Hb.	Erythrocytes	Barkan iron	Heilmeyer serum iron	Bilirubin
Mrs. B. B., aged 41	44%	4.2 Mill.	0	22 γ%	0.68 mg.%
Mrs. S. B., „ 45	35%	4.3 „	traces	36 γ%	—
Mrs. B. L., „ 50	38%	3.7 „	„	34 γ%	0.62 mg.%
Mrs. M. F., „ 43	43%	4.0 „	0	22 γ%	0.68 mg.%
Mrs. F. F., „ 35	38%	4.0 „ after repeated attacks of metrorrhagia			

A-iron = 0 γ%
B-iron = 30 γ%
C-iron = 90 γ%
D-iron = 60 γ%

Total Iron = 180 γ%

Mrs. N. H., aged 44. Hb. 52%. Erythroc. 4.0 Mill. Repeated metrorrhagia.

A-iron = 0 γ%
B-iron = 35 γ%
C-iron = 85 γ%
D-iron = 60 γ%

Total iron = 180 γ%

Therefore, in this form of anaemia the fractions A and B, i.e. the separable iron, vanish almost completely. These fractions include the iron of the pseudohaemoglobin which, originating from the haemoglobin, is in process of being converted into bilirubin. This reduction cannot be explained as reduction through physiological haemolysis, since the blood bilirubin is normal or even increased. *Heilmeyer* and *Anton* are of the opinion that this must be regarded as a selective reduction of certain forms of iron, which occurs gradually in the course of chronic loss of blood. The biologically more active iron, which is also more easy to separate, is especially indispensable for tissues which have been rendered highly anaemic. It is essential that they contain this special iron fraction for their own use in order to compensate for the difficult conditions of cell respiration which occur in anaemia. Hence the content of *Barkan* iron in the circulating blood is more greatly reduced than that of the iron which has been extracted by concentrated HCl according to the *Heilmeyer* technique. This view appears to be confirmed by the experiments of certain American authors who were able to note in severe, oft-repeated haemorrhagic anaemia in dogs only a slight decrease of the myoglobin content of the muscles, even after several months of severe anaemia. Thus the tissues are at pains to retain intact their iron and pigment content, at the expense of the circulating and depository iron. Similar observations have been noted in other forms of severe anaemia. Doubtless, in such cases, increased blood decomposition is the

evidence of a compensatory reaction, which takes the form of trying to set separable iron into circulation. But this problem will be treated at a later stage (Chapter VI).

In conclusion we wish to mention the observations of *Sachs*. He found that in haemorrhagic anaemia and even in the form induced by venesection (dog) the copper content of the blood increases, whilst that of iron diminishes. In this way the iron and copper curves intersect during the phase of bone-marrow regeneration. According to *Sachs*, this fact indicates the close co-operation between the metabolism of these two metals in blood regeneration. *Heilmeyer, Keiderling* and *Stüwe* made identical observations in the course of infectious anaemia.

(*b*) *Anaemia due to an insufficient supply of iron*

This form of anaemia may develop in the course of prolonged nutritional insufficiency, especially among the poorer classes or in invalids who do not follow rational habits of nutrition. But it occurs much more frequently as the result of a defect in iron absorption by the intestine. This is the function of the mucous membrane of the duodenum or of another section of the intestine immediately adjoining the stomach (cases of gastric resection or enterostomy; see also p. 15), which adapts the intensity of absorption to the needs of the organism; the iron absorption may therefore be retarded or even blocked, due to irritation or inflammation of the intestinal mucous membrane (enteritis), as well as to the consequences of certain forms of surgical intervention in the digestive tract (gastro-enterostomy and gastric resection). The gastric function is all-important in such cases, as we shall show at length, especially when we come to discuss idiopathic hypochromic anaemia. The gastric juice prepares the way for the separation of iron which is bound to organic complexes, without which it could not be absorbed. Moreover, it must convert this liberated iron by means of its hydrochloric acid content into the divalent form; in which form alone, according to *Starkenstein,* it can be take up through the intestinal wall. As iron absorption occurs principally in the duodenum, the simple fact of digestion taking place too quickly often suffices to bring about insufficient absorption. We give a few illustration of this:

T. M. N., aged 32. Good general health; for the past 2 months showed signs of debility. Complained frequently of vague digestive disturbances. Anamnesis showed no haemorrhage; menstruation regular, gastric acidity normal, but X-rays showed abnormally rapid passage through the gastro-intestinal tract. Hb., 51%; erythrocytes, 3.72 mill. *Barkan* iron, 23 γ%; *Heilmeyer* iron, 70 γ%. As a result of large oral doses of iron the absorption became good. After iron intake the *Heilmeyer* fraction showed values of 170 γ%. Intensive treatment *per os* for 2 months affected a cure.

Our second example is that of a patient, R.H., aged 60, who at the age of

58 underwent resection of two thirds of the stomach for prepyloric ulcer. The removal of the most important part of the stomach and the acceleration of gastro-intestinal transit caused a considerable insufficiency of iron absorption. Two months after the operation the patient was brought to hospital for severe, progressive secondary anaemia. Hb., 50% ; red blood cells, 3.6 mill. ; *Heilmeyer* serum iron very low, 19 γ%. The patient was given daily intravenous injections of iron, but only after a treatment of 32 injections, the last ten of which contained 12 mg. of iron each, could an improvement be noted. The haemoglobin rose to 66%, the red blood cells to 4.5 mill., serum iron to 58%. But a few weeks without treatment sufficed for the serum iron content to fall again to 13 γ%, resulting in anaemia, without any haemorrhage being involved.

The following are two cases of anaemia due to insufficient intake of iron, in which we determined the total serum iron and its four fractions:

Mrs. E. C., aged 50. Chronic gastro-enteritis with pronounced hypochlorhydria. Hb., 40 γ% ; erythrocytes, 3.8 mill.

A-iron = 0 γ%. B-iron = 60 γ%. C-iron = 60 γ%. D-iron = 30 γ%. Total iron = 150 γ%

Mrs. E. L., aged 62. Arteriosclerosis, malnutrition with incipient cachexia. Hb., 52% ; erythrocytes, 3.45 mill.

A-iron = 0 γ%. B-iron = 40 γ%. C-iron = 60 γ%. D-iron = 20 γ%. Total iron = 120 γ%.

The investigations of *Wakeham* and *Halenz* regarding the iron contents of various organs in experimental iron-deficiency anaemia in rats strike us as particularly interesting. These authors noted that the iron content of the muscles in this form of anaemia hardly deviated from normal. It diminished 25% in the myocardium, almost 50% in the kidneys and more than 50% in the liver, i.e., the organ which normally contains the greatest quantities of iron. The spleen showed practically no change.

These observations show the biological significance of the iron deposits in the liver, whilst those in the spleen remain outside the domain of physiologically active iron metabolism. In a condition of insufficiency, therefore, the iron does not show homogeneous diminution of its values in the various tissues. The iron of the muscle cell, i.e. that which is most important for the biocatalytic processes, remains almost unchanged, while the deposit iron from the liver and the circulating blood, by greatly diminishing its reserve supplies, is able to satisfy the demands of the muscular system.

In recent years we were able to observe a number of cases of anaemia associated with malnutrition (hunger conditions of the war), especially lack of meat; these cases also showed a low iron content in the serum. These forms of anaemia are often accompanied by hypoproteinaemia and hunger forms of oedema.

In five cases of severe hunger conditions we determined serum iron values of

15 γ%, 10 γ%, 28 γ%, 48 γ%, 18 γ%.

These anaemias are usually of a hypochromic type, although they show a certain tendency to the hyperchromic form if treated with iron only. But, if to this is added abundant protein in the form of meat, the anaemia will rapidly disappear as the general condition improves. We assume that this represents a complicated clinical picture in which there is deficiency, not only of iron substances, but also of protein substances and other biologically important substances (including vitamins).

(c) *Anaemia due to iron consumption for purposes other than haemoglobin formation*

This group includes the anaemias of the infectious diseases, neoplasms, growths and pregnancy. The problem of anaemia in infections leads to the consideration of iron metabolism outside the domain of erythropoiesis. The repeated observations of *Heilmeyer*, of other authors and of ourselves have shown that in the course of the acute illness there is a definite reduction of the non-haemoglobin iron content, without it being possible for the bone-marrow to co-operate in this mobilisation of iron; but there is an increased iron content in the reticulo-endothelial system. From this we must conclude with *Heilmeyer* that the iron is attracted by the reticulum, in order to co-operate as a stimulating agent in the fight against the infection undertaken by the organism. Part of it doubtless serves as material for the building up of the white blood cells. Another part, as we shall see on pages 194–6, must, in our opinion, act as bio-catalyst in the increased tissue oxidation produced by the fever. Here we therefore see in a particularly clear fashion that the clinical significance of the metabolism of circulating iron is not only restricted to the processes occurring during the changes in the blood, but applies also to a number of equally important processes connected with the living organism.

But it should be emphasised that the anaemia of infections is not only caused by a lack of iron due to iron mobilisation for other purposes, but also by a toxic action on the bone-marrow, as a result of which the passage of iron to this organ is also decreased owing to the arrest of erythropoiesis.

On pages 97 and 98, we mentioned the conclusions at which the American authors arrived, principally *Wintrobe* and his collaborators, *Whipple* and his school, to explain the anaemia of infection characterised by a decrease of non-haemoglobin iron and an increase of protoporphyrin in the blood. In order to explain this anaemia, we wish simply to recall here that one must evisage a qualitative and quantitative disturbance of the protein support of iron in the plasma, combined with the modifica-

tions of the iso-electric point of the proteins influencing the links binding iron in the plasma (*Neukomm*) and finally to an eventual disturbance of the formation of the globin of the blood pigment (*Wintrobe*).

The two following examples are concerned with the anaemia of infection:

(1) Mrs. R. H., aged 32. Chronic infectious arthritis, with protracted febrile periods; also anaemia with 65% haemoglobin and 3.89 mill. red blood cells. The *Barkan* iron showed a serum value of 26 γ%, the *Heilmeyer* serum iron a maximum of 112 γ%. In the course of the light sub-febrile periods the anaemia became a little worse, the haemoglobin content fell to values below 60%, and the number of red blood corpuscles to 3.5 mill. At the same time the *Heilmeyer* serum iron sank to 65 γ%. Nevertheless, the gastro-intestinal absorption of the metal was excellent in this case, as was shown by the curve of the *Heilmeyer* serum iron during oral administration of 1.0 g. ferri reducti:

Fasting	2 hours	4 hours	6 hours after peroral administration
78 γ%	150 γ%	116 γ%	115γ%

(2) Mrs. A. M., aged 40. Good general condition. Chronic cystopyelitis very resistent to treatments; periods of sub-febrile temperature. Deterioration of general condition and excessive debility. No haemorrhage noticeable, menstruation absolutely normal. No digestive disturbances. After 2 months the patient showed hypochromic anaemia with a marked deficiency of serum iron. The following are the results of the examinations:

	Hb.	Erythrocytes	Barkan iron	Heilmeyer serum iron	Reduction of blood cells
June 20	50%	3.9 mill.	traces	30 γ%	76 mm.
Sept. 27	55%	3.8 mill.	7 γ%	46 γ%	92 mm.
Sept. 30	61%	3.9 mill.	15 γ%	85 γ%	56 mm.
	Progressive abatement of condition of debility.				
Oct. 21	71%	3.9 mill.	8 γ%	102 γ%	45 mm.
Dec. 22	73%	4.6 mill.	25 γ%	94 γ%	21 mm.

Since the start of the observations the patient was subjected to a combined iron treatment, *per os* and intravenously; but not until the chronic infectious processes receded, as indicated by a less rapid decline of the blood, did the serum iron begin to rise and the anaemic condition to improve.

In two patients who showed hypochromic anaemia after a chronic infection we determined the total serum iron, with its four fractions.

Mrs. M. A., aged 26. For several years persistent chronic progressive pulmonary tuberculosis.

Hb., 42%; Erythrocytes, 3.8 mill.; sedimentation of blood cells, 21 mm.; A-iron = 0 γ%; B-iron = 70 γ%; C-iron = 90 γ%; D-iron = 110 γ%; Total iron = 270 γ%.

Mrs. G. E., aged 60. Chronic cystopyelitis.

Hb., 40%; Red cells, 3.6 mill.; sedimentation of blood corpuscles, 30 mm.; A-iron = 0 γ%; B-iron = 60 γ%; C-iron = 15 γ%; D-iron = 15 γ%; Total iron = 90 γ%.

In these two cases we could note a considerable reduction of the total serum iron, as shown in all the fractions, especially in those of the separable iron (A- and B-iron).

(d) Idiopathic Hypochromic Anaemia

Among the various forms of hypochromic anaemia, a type has been described in recent years which has been attracting the attention of physicians more and more, in view of the fact that it is frequently severe in form and shows particularly great resistance to treatment. This special anaemia has no clear aetiology and anamnesis usually yields no comprehensible explanations. Hence its name of " idiopathic or primary anaemia ".

Whether this corresponds to the *essential hypochromic anaemia* of *Scott* and *Bodley*, *Thiele* and collaborators, *Lundholm*, *Fowler* and *Barer*, *Scheid*, *Bickel*, *Morrison* and co-workers, etc.; the *primary hypochromic anaemia* of *Bode*, the *idiopathic hypochromic anaemia* of *Meyers*, or the *achylous chloranaemia* of *Karnelson*, *Heilmeyer*, *Mollow*, *Heinild*, *Rosegger*—the fact remains that the clinical picture is always the same: a number of definite symptoms, the cause of which cannot be ascertained. Some authors consider that this form of anaemia lacks a clearly defined character and hold that it represents merely a partial symptom associated with some primary pathological condition, such as avitaminosis or an internal secretory disturbance. Most writers. however, who have handled this form of anaemia describe it as a disease *sui generis*. This view is also supported by those workers who have disclosed in this condition certain constitutional hereditary factors and speak of a " constitutional hypochromic anaemia " (*Steiner*), a " familial ", or a " hereditary hypochromic anaemia " (*Lundholm*).

As a matter of fact, these authors have been able to examine a number of genealogical tables which showed that a fairly large number of the members of the family were affected by this form of anaemia. The same thing was found by *Thiele* and others. *Steiner* associates this anaemia with the blue sclera regularly found in such families, which fact led him to question whether these two symptoms might not be due to the same hereditary factors, or whether the optic symptom might possibly result from the anomaly of iron metabolism caused by the anaemia. *Lundholm* and *Micheli* attribute to heredity a role of prime importance in the pathogenesis of this hypochromic anaemia. *Naegeli* also mentions the complexity of the aetiological factors involved in producing this anaemia, of which the factor of heredity is doubtless the most important.

We prefer to designate this disease by the name " idiopathic hypochromic anaemia ". Its clinical picture is as follows:

The anaemia often shows pronounced anisocytosis with a tendency to microcytosis and leucopenia. It develops slowly, especially in women, in the forties, i.e. towards the end of ovarian activity. The patient complains of fatigue, gastric trouble, soreness of the tongue, which is always smooth and atrophic. Sometimes rhagades can be noted around the corners of the mouth; the nails are brittle, irregular, with deep grooves and longitudinal fissures. Paresthesae are often noted. Finally, *Micheli, van Goidsenhoven, Lederer* and others frequently observed splenomegaly which disappeared as the anaemic condition improved. The most important feature of this anaemia is gastric achylia, which is nearly always present and is the cause of this condition being called " achylic chloranaemia ". As a rule this achylia is not refractory to histamine.

Anamnesis often discloses menstrual anomalies (menorrhagia) and repeated pregnancies. *Meyers* emphasises the nervous disturbances incident to this disease: nervousness, anxiety, psychopathy, which in the opinion of this author represent part of a constitutional psychic sense of inferiority. Certain American authors believe that nutrition also plays a certain part in the appearance of this anaemia, since they observed this form particularly among the poorer sections of the population. *Leverton* and *Roberts* also attribute much more pathogenetic importance, in this form of anaemia, to iron deficiency in nutrition rather than to loss of blood from menstruation.

The observation of achylia has suggested a possible connection with pernicious anaemia. Actually, it has often been possible to diagnose the co-existence of pernicious anaemia and idiopathic hypochromic anaemia (*Kowalzig* and *Heilmeyer*) and also to observe cases in which pernicious anaemia develops on the basis of an essentially hypochromic anaemia (*Cotti* and *Balestrieri, Morrison, Swalm* and *Chevalier*). There are certain constitutional factors which are common to both these forms of anaemia. On the other hand, achylia can be held responsible for faulty iron absorption through the digestive tract and thus be considered one of the causes of hypochromic anaemia.

In addition to insufficient intake of dietary iron, it is assumed that there is also exceptional loss of iron during menstruation and pregnancy. In the latter case there actually occurs a great displacement of deposit iron from the placenta of the mother to the foetus. This mechanism explains the very high non-haemoglobin iron values found by *Guthmann* during pregnancy. According to *Scott*, only 50% of the circulating non-haemoglobin iron can

be restored by the mobilisation of the iron from the organs of storage. Hence, if the loss of circulating iron exceeds this quantity the organism will automatically be in a condition of iron deficiency.

Finally, the possibility must be entertained of an insufficiency of deposit iron at birth, which would lead to the assumption that there was a definite type of constitution with defective mineral metabolism, of which condition rickets, for instance, would represent merely one additional clinical manifestation of this pathological picture (*Thiele*). This hypothesis would be in accordance with *Vahlquist's* observation of the presence of hypochromic anaemia with pronounced hyposideraemia (10–42 γ%) which is frequently accompanied in children, especially up to the age of four, by achlorhydria and by skin and mucous membrane changes.

In the pathogenesis of this form of anaemia it is usual to assume that there is iron deficiency due to a lack of the gastric hydrochloric acid needed for the intestinal absorption of this metal. But these ideas will remain mere conjecture until a direct proof can be found that there exist certain changes or injuries of the intestinal mucous membrane which are specific for iron absorption. In order to explain this lack of iron absorption, *Thiele* reminds the reader that in this anaemia, independent of the achylia, there can always be noted abnormally rapid passage from the stomach to the small intestine. This view is based on the radiological observations of *Thiele* and *Pust*, which showed that while gastro-intestinal hypermotility is usually not found in either of the other forms of achylia, nor in chlorosis, it represents an invariable symptom of idiopathic hypochromic anaemia.

Finally, we consider it worth while to mention the observations of *Hallen*. In five cases of idiopathic hypochromic anaemia he found that the histamine-resistant achlorhydria vanished after systematic iron treatment. He therefore asked himself whether the achlorhydria was the cause of the faulty iron resorption or whether it was not itself caused by the iron deficiency. This query merits our fullest attention, since it may be associated with the problem of the presence of H-ions in the work of HCl formation in the gastric juice, as evidence of the mobilisation of the H-ions which are so closely bound up with cell respiration (*Jung* and others). According to this view the hydrogen conversion would be indirectly connected with that of important cell catalysts, including the conversions of iron and the vitamins of the B-complex. As a matter of fact, where there is an absence of the vitamin B-complex we often find achlorhydria (*Vannotti* and *Lang*), since the disturbances in cell respiration resulting from B-hypovitaminosis would presumably only permit an inadequate elimination of hydrogen in the form of gastric HCl. Iron, which likewise plays an important

part in the complex system of the bio-catalysts, might thus, like the vitamins of the B_2 group, also be connected indirectly with the mechanism of hydrochloric acid formation in the gastric juice.

The lack of HCl in the stomach is not necessarily the cause of faulty iron absorption in the intestine. This fact was noted by *Heilmeyer* in a case of histamine-refractory achylia, from which he concluded that in cases of achylous anaemia in which the serum iron content is not increased as a result of oral intake, the failure of iron absorption must be attributed to other factors, the seat of which is probably to be sought in the mucous membrane of the stomach or small intestine. In this connection we think it interesting to recall the experiments of *Lederer* relative to the digestion of dietary iron by the gastric juice. In the opinion of this author there exists, in addition to hydrochloric acid, an enzyme capable of disintegrating the dietary iron bound to other bodies—an enzyme which is no way connected with the anti-pernicious factor. In cases of idiopathic hypochromic anaemia, *Lederer* failed to find this enzyme, which is capable of converting iron into the ionised form, in the gastric juice.

In addition we wish to draw attention to the important role played by ascorbic acid in iron absorption in the duodenum. As we mentioned in the preceding chapter, vitamin C would prevent oxidation of the reduced iron which has been prepared for absorption and would therefore constitute an important factor in connection with uptake of iron by the intestine.

We must also mention here what we said on page 17 concerning the action of para-aminobenzoic acid in iron metabolism (*Vannotti* and *Kalbermatten*) and its failure in certain cases of idiopathic hypochromic anaemia.

Defective iron absorption, inadequacy of the deposit iron and an exaggerated loss of iron by the organism—these, therefore, are the chief factors in this hypochromic anaemia. The expression of this reduction of depository iron and of the circulating metal is found in the determination of the non-haemoglobin iron in the serum and plasma. Actually *Heilmeyer* and *Plötner* were able to observe in a number of cases of idiopathic hypochromic anaemia a very low value of the residual serum iron—a value that, even after iron administration *per os*, did not even show a temporary rise.

Finally these authors failed to observe any change in the mechanisms of regeneration and destruction of the blood corpuscles. As a matter of fact, the urobilin formation and its quotient appeared even to be raised. *Heilmeyer* and *Plötner* wondered why the organism did not utilise the iron which was liberated at the time of bilirubin formation in order to form new haemoglobin.

These authors found no satisfactory answer to their question. Nevertheless, we are of the opinion that one does exist. But before proceeding to a discussion of these particular facts, including the general problems presented by essential hypochromic anaemia, we will give two personal observations of particularly acute cases of this form of anaemia.

(1) Miss E. M., aged 33. Anamnesis. Rather delicate as a child. Had severe influenza at 12 years of age (1918), followed by a slow convalescence; could not remember having had any diseases of childhood. At 14 started normal menstruation. From 18 to 19 sub-acute digestive disturbances, loss of appetite, often nausea and vomiting. There was talk of anaemia, various physicians were consulted and successive treatments were given of HCl and iron *per os*, sometimes with temporary success. When interviewed the patient complained of excessive fatigue, palpitation and shortness of breath after exertion: of paresthesiae, lack of appetite with vomiting, occurring apparently from no definite cause. The patient was capable of performing regular office work, but reacted particularly badly to an excessive amount of work. Had never had severe haemorrhages. Menstruation regular, even though sometimes abundant. Patient did not mention having had any infectious disease other than the influenza of 1918. Family anamnesis: Mother operated on for exophthalmic goitre. Father and one sister in good health.

On examination: General condition comparatively good. Weight 53 kg., height 161 cm. Face pale, conjunctiva and mucous membrane of mouth pale. Sclera bluish. Deep rhagades at corners of mouth. Moist tongue, relatively slightly coloured, not atrophic, slightly coated. Tonsils and lymphatic glands normal; slight splenomegaly. Liver, heart and lungs normal. Abdomen: anacidic gastritis with, however, slight secretion of HCl after histamine. Intestines, kidneys, sex organs normal. Limbs: the nails were brittle, their surfaces irregular, they split both longitudinally and transversely, which sometimes gave their free edges a serrated appearance. Nervous system: Slight paresthesiae, especially in the face and extremities. Apart from this, nothing special noted. Urine: protein = 0, sugar = 0, urobilinogen not increased, urobilin normal, coproporphyrin = traces, sediment, a few leucocytes and epithelial cells. Stool: benzidine negative, no intestinal parasites. Rapidity of blood cell sedimentation, 1.5 mm.; WR negative; bilirubin = 0.34 mg.%. *Barkan* iron in serum, traces; *Heilmeyer* serum iron, 19 γ% haemoglobin, 48% erythrocytes, 4.5 mill.; leucocytes, 3.700; neutrophiles, 59.5%; lymphocytes, 36%; large monocytes, 4.5%; reticulocytes, 2%.

Treatment with iron *per os* had to be suspended since, despite the simultaneous administration of HCl, digestive disturbances supervened. Intravenous iron treatment not very successful. The *Barkan* iron, nevertheless, attained 10 γ%, and that of *Heilmeyer*, 85 γ%. Cessation of condition of weakness. Combined intravenous treatment with iron and Campolon caused the haemoglobin to rise temporarily to 50%. And even after iron treatment lasting for 4 months the haemoglobin value remained around 50%. As the *Heilmeyer* serum iron was at about the normal value we proceeded to carry out blood transfusion. This caused vomiting, lasting 48 hours, which could not be stopped. As a result of the transfusion, the *Barkan* iron rose a little during the first few days, probably as a result of slight haemolysis, as could be seen from the increased bilirubinaemia and the somewhat retarded increase of the *Heilmeyer* iron. After 4 days the first indications of a return of haematopoietic activity was seen in the increased haemoglobin and red blood cells with a corresponding reduction of the serum iron content. This lasted only a short time, but from then on the blood values were maintained. Next, menstruation came on and, for the

time at least, this did not appear greatly to affect the iron metabolism. Although it was fairly abundant and lasted 5 days, it was followed by hardly any perceptible decline of the serum iron content, or of the other blood values. The patient felt comparatively well, and resumed her work. But in the course of the next month we observed a slow and progressive deterioration of her general condition, accompanied by a reduction of the serum iron content. Menstruation brought a definite reduction of the haemoglobin and red blood cell values, which had been maintained at 47–48% and 5 million, respectively. The slow progressive iron deficiency, which followed as soon as the patient ceased to receive treatment, appeared therefore to be a determining factor in this case. This deficiency was to be explained, in part at least, by the unfavourable effect of the gastric anacidity on the iron absorption, as is proved by the fluctuations of the serum iron content after oral administration of reduced iron alone, and with HCl:

	Fasting	2 hours	4 hours	6 hours
Reduced iron per os	76 γ%	70 γ%	78 γ%	74 γ%
Reduced iron + HCl	93 γ%	127 γ%	121 γ%	98 γ%

The table below gives a summary of the chief blood analyses conducted during the treatment:

	Hb. %	Erythrocytes Mill.	Leucocytes	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Mar. 5	45	4.7	3500	0	19	0.50
	Condition of debility; intravenous iron treatment.					
May 21	40	4.6	3500	0	45	0.34
July 10	Combined iron treatment *per os* and intraven. and Campolon.					
	42	4.5	3600	0	87	0.60
	Blood transfusion followed by uncontrollable vomiting lasting 3 days					
July 10	43	4.8	4500	Traces	85	0.70
„ 13	43	4.95	4600	15	98	0.71
„ 15	50	4.88	3300	0	63	0.88
	Menstruation 5 days.					
„ 20	47	4.53	3700	15	100	0.55
„ 26	48	4.2	5000	15	88	0.55
Aug. 1	46	4.2	4000	0	100	0.44
	Patient feels comparatively well and resumes her work.					
Sept. 20	48	4.5	6600	3	70	0.54
Nov. 4	47	5.02	3800	8	60	
„ 12	Immediately after menstruation lasting 5 days.					
	40	4.5	4200	18	52	
	6 months later.					
	48	4.74	4700	10	50	0.34

The fractional iron determination gave the following results:

A-iron = 5 γ%
B-iron = 45 γ%
C-iron = 125 γ%
D-iron = 135 γ%

Total iron = 310 γ%

(2) A. E., aged 40. Very delicate even as a child, with pallor and digestive disturbances; much vomiting and constipation. Menstruation at 16, irregular, tending to be abundant. Between 18–20 years of age, although

her general condition was good, patient showed very pronounced pallor and had great difficulty in breathing after exertion. The family doctor diagnosed chlorosis. The pallor gradually vanished and during the next few years the patient felt better, although menstruation continued to be very abundant and patient had continual digestive disturbances. Exacerbation of dyspeptic troubles since her thirties; frequent menorrhagia.

The present examination showed non-histamine-resistant achlorhydria and a fibromatous uterus with slight menorrhagia. On the whole the general condition was poor, with a weight of 42 kg. Blood condition: haemoglobin 38%; erythrocytes, 3.4 mill., with anisocystosis and slight poikilocystosis and polychromasia. The following are the blood values during treatment:

	Hb. %	Erythro-cytes Mill.	Leuco-cytes	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Sept. 19	38	3.4	3900	Traces	60	0.56
„ 20	38	3.8	4100	Traces	77	1.55
	After slight menorrhagia:					
Oct. 26	34	3.6	3600	Traces	37	1.20
	Intensive iron treatment *per os* and intravenously.					
Nov. 21	33	3.7	8200	Traces	90	0.71
Jan. 9	Sub-total hysterectomy.					
	Iron treatment *per os* and intravenously.					
May 20	61	4.2	8100	10	82	0.41

We found in this patient the phenomenon of increased haemolysis, which, if the organism suffers great deficiency, has the effect of supplying iron direct either to the tissues or to the bone-marrow. We also found very low *Barkan* iron values, corresponding to increased bilirubin content. This fact is all the more conspicuous in this case, since the maximal values of bilirubinaemia correspond to the lowest values of *Heilmeyer* serum iron and to a period of very great debility.

These cases of hypochromic anaemia only react after a particularly lengthy treatment with iron. It may even happen that as a result of iron treatment a normal serum iron content may be re-established some time before blood regeneration is resumed. In this case, for instance, a *Heilmeyer* serum iron of 90 γ% corresponded to a haemoglobin content of 33%. This condition had, moreover, existed in the same patient on two previous occasions after prolonged menorrhagia.

(3) Mrs. B. B., aged 45. Anamnesis: As a child she was pale and delicate, although she was able to attend school regularly. Mild diseases of childhood without complications. Menstruation at 12 years of age, two or three times, after which it ceased until she was 17. From then on regular menstruation, sometimes very abundant, particularly as she approached the forties. Four normal pregnancies, well supported. Sometimes vomiting without any apparent cause. Patient showed slight resistance to fatigue. At 41 (1935) had to go to hospital for secondary anaemia and pyelitis. In 1938 second hospital treatment for hypochromic anaemia and gastric anacidity; not much improvement. In the same year again in hospital and

also in 1939 for the same hypochromic anaemia; each time only moderate results. Family anamnesis: Father died of gastric cancer at 71; mother pale and delicate like patient herself; died at 61 of heart disease. Had no brothers or sisters. A relation also suffered from anaemia.

On examination: General condition comparatively good. Weight 56.6 kg.; height 160.5 cm. Facial skin pale, only slightly mobile; sclera bluish. Skin waxen; painful rhagades at corners of mouth; pale mucous membranes, tongue flat and shiny, slightly yellowish; often has sore tongue; small goitre; lungs normal; heart normal, occasional tachycardia. Slight tremor of extremities, but unaccompanied by any optic symptom of exophthalmic goitre. Histamine-resistant achlorhydria. Liver and spleen not enlarged, not painful upon palpation. Sex organs normal; limbs comparatively small; hands long; abnormally brittle finger nails, irregularly striated in a longitudinal direction. Paresthesia and a sensation of tingling in hands, feet and at the flexor surfaces of the elbows and knees. Urine normal. Stool: Benzidine negative, no intestinal parasites. WR = 0.

Here is a summary of the analyses:

		Hb. %	Erythrocytes Mill.	Leucocytes	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
May	8	42	4.0	4500	10	24	0.61
		Condition of debility, menorrhagia. Treatment: Sistomensin, iron intravenously.					
June	7	30	3.4		0	30	0.66
		The haemorrhages stop. Combined treatment with iron *per os* and intravenously plus Campolon. The state of debility is much less pronounced.					
July	20	34	3.5	7100	Traces	65	0.29
		Uninterrupted vomiting.					
Aug.	25	Transfusion of 250 cc. blood.					
„	26	40	3.7	5200	Traces	40	0.61
„	28	40	3.7	5700	Traces	22	0.83
Sept.	3	43	4.0	5700	Traces	59	0.82
„	23	46	4.3	5300	Traces	90	0.61
		Campolon treatment.					
Dec.	15	46	4.3	6000	Traces	90	0.60
		Six months later:					
		48	3.36	6200	Traces	65	0.10

Fractional iron determination:

A-iron = 5 γ%
B-iron = 60 γ%
C-iron = 120 γ%
D-iron = 175 γ%

Total iron = 360 γ%

The most obvious thing about these observations is, first, the fact that the haemoglobin content (irrespective of the treatment applied) never rose to normal values; secondly, that the intensity of the functional disturbances (debility, etc.) seemed to follow the serum iron values. Thus, the iron metabolism was considerably altered. But the fact that as a result of treatment it was gradually possible to restore a normal serum iron content, without greatly increasing the haemoglobin content, clearly shows

that the iron metabolism alone was not the cause of this pathological picture. However, the poor therapeutic results obtained from the blood transfusion renders it highly improbable that a deficiency of anti-anaemic elements (Chromogen) other than iron was involved in the aetiology of this anaemia. The whole clinical development, the persistence of a particularly low haemoglobin value which failed to respond to therapeutic treatment, the frequent vomiting that could not be checked—all these symptoms lead us to assume that we were here confronted with a constitutional defect of the central regulatory system affecting at the same time the haematopoiesis and the general iron metabolism.

Our observations show, as is usually the case, pronounced reduction of the *Heilmeyer* serum iron, but a still greater decline of the *Barkan* iron. Even after heavy iron administration, oral or parenteral, these two iron values remained low for a long time. Finally, it is interesting to note that the value of the non-haemoglobin iron, in contrast to that of the case above described, often remains diminished, even in the less obstinate cases, and even after an improvement in the condition of the blood has taken place.

The fractional determination clearly shows that the total serum iron is not necessarily greatly reduced, as might have been thought from the determination of the *Barkan* and *Heilmeyer* fractions. The value of the total iron is here at the lower limit of normal; the fractions of the non-separable iron and of the iron of the protein precipitate are normal; only the separable iron, that is, the biologically most important iron, is greatly decreased. This fact would explain the appearance of several general symptoms as being the expression of a definite deficiency of the iron indispensable to maintain the chemical processes of the cells. It must therefore be asked what may be the cause of this difference in the distribution of the iron fractions—a difference which we already observed in hypochromic anaemia as the result of chronic loss of blood.

The bilirubin is normal, sometimes even slightly augmented. There are many signs of normal or even increased destruction of haemoglobin. This fact had already attracted the attention of *Heilmeyer*, who assumed that there are abnormally fragile blood corpuscles in this form of anaemia. He considered that the condition was one of a fundamental change in the formation of the blood cells due to iron deficiency. By supplying this metal a speedy improvement of erythropoiesis would result and would be revealed chiefly in the number of reticulocytes. Heilmeyer believed that in such cases the effect of iron is equivalent to that of a vitamin.

In a case of idiopathic hypochromic anaemia in which

iron was administered by mouth, *Heilmeyer* noted that the values of the serum iron remained absolutely unchanged, whilst in normal individuals they often begin to rise after two hours. However, this behaviour of the iron after administration in achylic anaemia is not constant, as the author and we ourselves were able to observe. On the other hand, the iron balances determined by various other authors do not appear to confirm the hypothesis of defective iron absorption through the intestine. *Reinmann, Fritsch* and *Schick*, in their analyses of the iron balance in various forms of anaemia, observed that in idiopathic hypochromic anaemia the organism is able to take up 50% of the iron administered *per os*. (In contrast the absorption in the case of normal individuals is nil.) This retention occurs very rapidly, as early as the first day after intake. *Fowler* and *Barer*, who studied the aetiology of this anaemia with the help of balance tests, found that these patients showed considerable iron retention after administration.

We can confirm this iron absorption, excellent in some cases, in idiopathic hypochromic anaemia. Here, for instance, is the curve of the *Heilmeyer* serum iron after administration *per os* of 1 g. of ferri reducti:

M. K. D., aged 46. Idiopathic hypochromic anaemia.

0	2 hours	4 hours	6 hours
52 γ%	170 γ%	365 γ%	208 γ%

and as a comparison, the curve obtained in the case of a normal individual after the same administration of iron:

0	2 hours	4 hours	6 hours
121 γ%	132 γ%	126 γ%	114 γ%

The experiments in iron administration of *Fowler* and *Barer* do not permit us to assume that there was any deficiency in the iron intake in this type of anaemia, although they indicate that the inadequate clinical improvement did not correspond to the comparatively pronounced iron retention. This difference is probably due to the fact that the absorbed iron served the dual purpose of restoring the reduced deposit iron and of forming haemoglobin.

Fowler and *Barer* also occupied themselves with the problem of the utilisation of the iron given parenterally. They noted what might be called a total iron retention; but the haemoglobin and red blood cells did not show the calculated increase that was expected —a circumstance which indicates that the iron injected was used for other purposes. On the other hand, only through oral administration could an improvement of the anaemic condition be achieved in the long run. The iron could only be utilised for

erythropoiesis once it had been administered in considerable quantities; otherwise the possibility is not excluded that the oral route is more effective than parenteral administration. Hence we are unable to obtain a uniform picture of iron metabolism in this form of anaemia, as we are confronted by a number of different aetiological factors. Actually we were able to observe cases in which a small administration of HCl sufficed to improve greatly the intestinal absorption of the iron, and other cases in which only a protracted treatment with iron was able to produce a normal serum iron level. On the other hand, the disturbance of iron absorption through the intestine is not a constant phenomenon and can therefore not be designated as characteristic of idiopathic hypochromic anaemia. In this form of anaemia the inorganic iron salts (ferrum reductum) are usually resorbed, which explains the good therapeutic results obtained as a result of intensive and continuous treatment with iron. But it would appear that nutritive iron, i.e. the iron which is associated with organic molecules or bound up with organic complexes, has great difficulty in penetrating the intestinal wall in this form of anaemia.

We are more and more interested in the concept of *Lederer*. He believes that in this particular form of anaemia the gastric juice lacks the enzyme needed for splitting off the iron from the organic complexes of the food. The ionised iron, given in the form of a medicament, which is not absolutely dependent upon the *Lederer* enzyme, can act directly. This hypothesis would admirably explain the observation of our patient, M. K. D., whose curve of iron resorption after administration we have given above. The continuous administration of reduced iron gradually brought about a cure of the anaemia:

	Hb. %	Erythrocytes Mill.	Reticulocytes %	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Oct. 12	50	4.3	2	15	52	0.40
Rp. ferri reducti per os.						
Oct. 28	56	4.8	24	22	51	0.66
Nov. 6	60	4.9	15	14	67	
Nov. 16	68	5.1		24	135	1.18
Nov. 27	78	5.3		42	108	
Dec. 17	82	5.4		28	90	0.75

The lack of therapeutic success resulting from giving the iron via the parenteral route would, according to *Lederer*, be attributable to the administration of inadequate amounts of iron, these being insufficient to satisfy the demands of an organism impoverished of this metal, whether for the purpose of restoring

the empty deposit supplies, or to compensate for the lack of iron in the tissues and the greatly affected haematopoiesis.

We have therefore come to the conclusion that the inadequate iron absorption in idiopathic hypochromic anaemia applies without any exception only to organic iron in food. The causes of this inadequacy are well known to us: they may be either a lack of hydrochloric acid and of a special enzyme (*Lederer*), or too rapid a passage of the food into the small intestine (*Thiele*), or finally a lack of vitamin C in the small intestine.

If, now, we consider the pathogenesis of this form of anaemia in the light of the works of *Starkenstein* and *Weden*, of which we have already made detailed mention in the first section of this book, we are led to the following interpretation: If absorbed in a reduced form, this iron, which is particularly well adapted for biocatalytic processes, circulates for a long time in the normal organism before becoming deposited in the organs which need it. This iron is slowly oxidised; in other words, it gradually passes into the trivalent form and ceases to be active. As a result, it loses its catalytic power and reaches the organs of storage in an oxidised form. Finally, the reticulo-endothelial system of the liver can again reduce this oxidised iron, that is, convert it from trivalent into divalent inactive iron, for the purpose of building up the haemoglobin molecule. A great deal of the iron that has been freshly absorbed by the intestine possesses catalytic properties, hence it is iron which is principally involved in the chemical exchanges, and particularly in tissue respiration. Therefore, a reduction of intestinal absorption of the nutrient iron affects most heavily and directly the iron involved in the function of tissue catalysis. This would produce a deficiency of the iron which is closely associated with the needs of cell respiration, thus bringing about a reduction of the active iron of the tissues (respiratory enzymes), and what might be termed a *peripheral anaemia of the tissues*—a condition, of course, closely allied to anaemia of circulation. The latter state is not far-reaching, functionally considered. It appears that the reduction of the value of the separable circulating iron chiefly impedes the functioning of the cell. The organism mobilises this iron in order to transport it to the cell, whilst the non-separable iron which does not possess the properties of active iron remains in the blood stream and still shows approximately normal values in this form of anaemia. In the face of an urgent need of biologically active iron, for the purpose of repairing an anaemia of the tissues which might have serious consequences for the life of the cell, it is probable that the iron demands of the bone-marrow are only satisfied secondarily. This is the reason why we see the development of hypochromic

anaemia due to a lack of iron, since the biologically active iron is claimed by the tissues for their normal functions. The reduction of the active tissue iron, for its part, leads to a lack of that oxidised fraction which, proceeding from the tissues, should be prepared by the reticulum in order to be built up into inactive trivalent iron, that is, into iron needed by the bone-marrow to construct haemoglobin. In order to meet this serious situation the organism maintains normal haemolysis, which is increased as compared with the blood's content of red blood cells and haemoglobin, so that the iron may be supplied direct either to the tissues or the bone-marrow. But haemolysis takes place only in the liver and not in the circulating blood, which in this case, anyhow, is poor in blood pigment and blood iron. This, in our opinion, partly explains the normal content of bile pigment and the extraordinarily low value of the *Barkan* iron.

Idiopathic hypochromic anaemia would therefore not merely be the expression of a reduction of the iron needed for haemoglobin formation, but also of a lack of iron endowed with catalytic properties. We should, therefore, be confronted with an anaemia of the tissues which would in part explain certain typical symptoms accompanying this illness.

Heilmeyer and later *Waldenström* attributed the appearance of trophic disturbances of the nails, dermal phenomena, rhagades at the corners of the mouth, to lack of iron. These authors and we ourselves observed a more or less rapid disappearance of these alterations after systematic treatment with iron. We noted and described the same symptoms in patients showing pronounced hypovitaminosis B_2; hence we regard them as a typical evidence of vitamin deficiency. These same pathological manifestations also disappear without iron administration after lactoflavin and nicotinic acid have been given. Now how is this conformity to be explained? In many cases, when studying urine elimination before and after vitamin administration, we observed obvious hypovitaminosis B_2 in idiopathic hypochromic anaemia. Biologically considered, iron, lactoflavin and nicotinic acid are agents of considerable significance in connection with the function of cell respiration. As stated, iron plays a most important role in the chemical processes of the cell, and lactoflavin is the most important constituent of the respiratory yellow enzyme. Thus these two substances are frequently in close functional collaboration. It is therefore not excluded that an absence of catalytic iron creates a great need on the part of the body for vitamins B_2 and PP., in order to compensate in anticipation for the possible functional disturbances in cell respiration. With this phenomenon of hypovitaminosis B_2 might possibly also be associated gastric

achlorhydria (hypothesis of *Jung* concerning the function of the hydrogen iron liberated by tissue respiration, as a constituent factor of gastric HCl). Hence there is a tendency to assume that the absence of one or the other of these two elements which are indispensable for normal cell oxidation produces the same clinical symptoms, i.e. superficial alterations in the skin and trophic disturbances of the finger nails. These alterations are a clinical proof of the significance of these two factors in cellular metabolism.

For these conditions, which are based on a lack or insufficiency of biological catalysts for the regulation of cell respiration and cell metabolism, *Vannotti* has described a particular clinical syndrome: "the syndrome of insufficiency of bio-catalysts."

It might be asked whether other forms of anaemia, especially the severe forms provoked by intense haemorrhage, are also accompanied by such an anaemia of the tissues. The loss of great quantities of blood entails an impoverishment of all the iron fractions in circulation, including part of the catalytic iron. This would, therefore, not be a case of one-sided impoverishment, similar to that which must be assumed to represent a principle in hypochromic anaemia. Observations on the iron balance after administration have shown in a number of cases of anaemia of a varied aetiology that there is often a retention of 25 to 35% of the iron given *per os*, without this being followed by any visible increase of the haemoglobin content.

It is remarkable that this form of anaemia often fails to react to iron therapy, or at least in only an inadequate manner. This lack of therapeutic success is partly to be attributed to the fact that the faulty intestinal absorption of iron acts as a check in oral administration and that, on the other hand, parenteral iron therapy introduces insufficient quantities of iron. As a matter of fact, the organism needs great amounts of iron in order to overcome the iron deficiencies of the tissues and organs of storage, which will then place the iron at the disposal of the bone-marrow. Moreover, there is always a possibility that the iron which comes into circulation via the parenteral route is not in a physico-chemical form which is adapted to take part in iron metabolism, as is the case if the iron passes through the intestinal wall.

Finally, it is conceivable that the production of idiopathic hypochromic anaemia is based on a lack of other important substances and catalysts, as suggested above. Thus it is interesting to note that in certain cases where simple iron therapy has failed, success can be attained by a combined treatment with iron and certain vitamins of the B-group (above all lactoflavin and nicotinic acid), and also by yeast extracts.

Moreover, it must be asked whether this form of anaemia is

not a manifestation of protein deficiency, in the sense of *Whipple's* aminoacid-deficiency anaemia. It is certain that in many cases the anaemia can be definitely ameliorated by repeated small transfusions.

To conclude this section we wish to emphasise the importance of the hypothesis of *Alsted*, who considers that chlorosis is essentially a form of juvenile anaemia, with reduced serum iron. In seven cases of typical chlorosis examined by this author he noted a very clear reduction of the serum iron content. In addition to these symptoms, disturbances of digestion and menstruation, as well as excessive general fatigue, were regularly observed. However, after washing out the stomach the gastric acidity was found to be normal. According to this author chlorosis is distinguished from essential achlorhydric anaemia by the absence of achlorhydria. This form of idiopathic juvenile anaemia with reduced serum iron content does not, according to *Alsted*, show any symptoms that would explain an anaemia, and above all a reduced serum iron content. This form of anaemia reacts favourably to iron treatment. Its aetiology is still vague but, as has already been stressed by many authors, the constitutional factors, general hygiene, nutrition and the endocrine disturbances must be considered as the most important causes of the development of chlorosis.

(*e*) *Hypochromic anaemia produced by iron deficiency as a result of several causes*

The different causes of iron deficiency in the organism may be associated in the same patient in varying proportions, thus producing obstinate forms of hypochromic anaemia which are often very hard to treat.

The multiplicity of the factors accompanying iron deficiency in the pathogenesis of these anaemias with lowered serum iron content and their effects on the haemoglobin content, the red blood cells and the serum iron is clearly illustrated in the following illustrations (*Delachaux*):

C. C., aged 33. Enjoyed good health until 1936. That year she had to have all her upper teeth extracted. Patient did not wear a denture and since that time suffered from loss of appetite. At the end of 1936 casual discovery of a positive Wassermann. Combined treatment with Neosalvarsan-Oleo-Bi; two treatments very well supported; negative Wassermann since July 1937. In July 1937 premature birth with very great haemorrhage. No gynaecological complications resulted, but the patient was never restored to complete health. She remained pale, tired quickly and complained of headache, dizziness, palpitations and cramps in the calves after the slightest exertion. In May 1938 she came to the Polyclinic for the first time, after having previously taken in succession Campolon, iron with hydrochloric acid, and Redoxon. Haemoglobin, 32%; erythrocytes, 2.5 mill.; anisocytosis, polychromasis, poikilocytosis. Colour index: 0.68. Leucocytes,

2300, of which 65% were neutrophile polynuclears, 30% lymphocytes and 5% monocytes. Blood cell resistance, 0.40–0.20. Non-haemoglobin serum iron 25 γ%. A general examination revealed no abnormality, with the exception of slight hypochlorhydric gastritis. No blood in stools. Liver and spleen normal. No haemorrhagic diathesis. WR negative.

We now give the curves of the haemoglobin and non-haemoglobin iron values and the number of red blood cells during treatment:

	Hb. %	Erythrocytes Mill.	Iron γ%	Weight kg.	Treatment
May 15	32	2.5	25	57.0	Ferri reducti + acidol-pepsini + Redoxon + Campolon
June 1	35	2.6	25	57.5	
„ 10	34	2.6	20	58.4	
„ 20	54	3.5	14	57.4	Inhepton
„ 30	55	3.5	18	57.5	
July 7	65	4.2	18	57.5	Ferri reducti + Redoxon
„ 14	65	4.2	20	57.5	
„ 21	70	4.6	20	57.2	Ferri reducti + Campolon
„ 30	70	4.5	25	57.2	
Aug. 15	78	5.3	20	59.0	Ferri reducti + ac. arsenicosi
„ 30	78	5.3	30	59.4	
Sept. 30	75	4.8	45	59.8	No treatment
Oct. 30	85	5.0	85	60.4	

Briefly summarised, this was a case of a debilitated erythropoietic system due, in the first place, to chronic intestinal disturbances, since faulty intestinal absorption entails a serious deficiency of iron and very probably also a lack of other substances absolutely indispensable for the normal formation of erythrocytes; secondly, due to the existing lues and the resulting treatments with salvarsan and bismuth which, as is known, often affect the bone-marrow in an injurious manner. Weakened, but still able to maintain a normal blood picture under conditions of normal life, this blood-forming system could no longer withstand the haemorrhage of July, 1938.

It is clear that in this case the anaemia was not only caused by an iron deficiency, which is revealed by the reduction of the non-haemoglobin iron from 90 γ% to 25 γ%, but also by the inertia of the bone-marrow. As the iron and liver extracts when given separately had no influence on the anaemia, it must be assumed that the balance in erythropoiesis which was required was only attained by their therapeutic co-operation. As a result of the combined treatment (iron plus liver extract plus Cu plus Mn) the

non-haemoglobin iron content declined in spite of the iron administration. This reduction of the iron value, corresponding to an increase of the haemoglobin value and the number of red blood cells, is evidence of the resumed functioning of the blood-forming system (*Heilmeyer, Vannotti*) in view of the fact that the accelerated elaboration of the haemoglobin and the red blood corpuscles was inevitably accompanied by considerable iron consumption. If the organism has not sufficient reserves at its disposal, the iron begins to run short and for lack of the basic substances erythropoiesis is quickly paralysed. To this lack of iron, resulting from the active resumption of blood formation, is to be attributed in part the rapid cessation of the therapeutic effect in our patient.

Once the inertia of the blood-forming system was rectified the pathogenesis of this complicated anaemia was reduced for a time to simple iron deficiency. We therefore considered that greater quantities of iron were indicated. As a result of this treatment the haemoglobin again rose to the value of 65%, whilst the non-haemoglobin iron slowly increased again to its initial level of 25γ%. At 65% the haemoglobin value remained stationary, whereas that of the non-haemoglobin iron continued to rise. This second stop in the haemoglobin curve must therefore now be ascribed less to iron deficiency than to an arrest in the blood-forming system. Accordingly we combined the liver extracts with the iron therapy and observed a new rise of the haemoglobin curve.

Our patient showed considerable iron deficiency. At the end of treatment the number of red blood cells showed a clear tendency to decrease (from 5.3 mill. to 4.8 mill.)—a thing which is entirely comprehensible if the value of the non-haemoglobin iron is taken into consideration, which at that time had only reached 45 γ%, i.e. approximately half the normal value. Despite the continuous administration of assimilable iron, the non-haemoglobin curve only showed definite signs of rising after a few weeks; it attained physiological values fairly long after the haemoglobin had reached its normal content. Later, however, and without the aid of any kind of therapy, the values of both haemoglobin and red blood cells remained normal. Thus we can only consider the anaemia cured from the time that the iron reserves of the organism were completed, i.e. when the serum iron content and the values of the haemoglobin and red blood cells were also normal. As long (in iron-deficiency anaemia) as the serum iron remains low, the blood-forming system does not recover its stable functional balance, and there will always be many possibilities of relapse.

2. *The anaemias with normal or increased blood iron and serum iron*

Certain forms of anaemia show a normal serum iron content; therefore their aetiology does not appear to lie in iron deficiency. They may be hypochromic, normochromic or hyperchromic in form. In the first case the reduction of haemoglobin formation is not due to an absence of the metal, but of another constitutional element of haemoglobin. On the other hand, the existence of hypochromic anaemia with a normal serum iron level can only be explained by the functional inactivity of the bone-marrow. There are innumerable clinical observations of anaemia following upon infection or intoxication which indicate diminished activity of the bone-marrow.

An abnormally high serum iron content in one case of anaemia may be the expression of increased destruction of red blood cells. As we have already seen, haemolysis releases iron, which passes from the blood cells into the plasma or serum. This increased serum iron content is found in acute attacks of both haemolytic and pernicious anaemia.

The hyperchromic anaemias belong in general to the group of anaemias with increased serum iron, in which we include, besides the aregenerative forms and certain anaemias showing toxic interference with the bone-marrow, also pernicious anaemia with its sub-divisions, the aplastic hyperchromic anaemias, the leucaemias, the thrombopoenias and panmyelophthisis, in all of which *Heilmeyer* demonstrated a normal or increased serum iron content. Below we illustrate the various types of this group of anaemias with the aid of clinical observations.

(*a*) *Iron-refractory hypochromic anaemias*

Haemoglobin is composed not only of iron, but also of a porphyrin ring and a globin. As is known, the porphyrin ring consists of four pyrrole nuclei. Hence the body must synthesise the chromogens of the blood pigment from the pyrrole nuclei in order to be able to furnish the bone-marrow with the porphyrin needed for haemoglobin formation. Some of these pyrroles probably originate from the haemoglobin liberated during haemolysis or from bilirubin; but the direct participation of these pigments in haematopoiesis cannot be very great, since it is known that the parenteral administration of haematin leads to more extensive elimination of the derivatives of bilirubin (urobilin and stercobilin), without haematopoiesis being affected in the slightest degree.

One is therefore led to assume that the body is capable, at least to a limited extent, of synthesising the important porphyrin ring from simple pyrrole bodies introduced with the food. The

experiments of *Fontès* and *Thivolle* in animal experiments have shown that as a result of the elimination of tryptophane and histidine from the normal diet death results, due to severe anaemia. Thus the healthy organism would appear to synthesise the porphyrin ring of the haemoglobin with the help of relatively simple pyrrole bodies, such as tryptophane, which is one of the few nutrient substances which contain the pyrrole nucleus. Similarly, it appears to build up globin with the aid of aminoacids. *Fontès* and *Thivolle* and later *Whipple* were able to secure good results in certain cases of anaemia by parenteral administration of tryptophane, histidine and leucine.

On this subject, it is interesting to point out the fact mentioned by *Madden, Whipple* and their collaborators: "Leucine, isoleucine and tryptophane deficiency in the dog causes a decline in plasma protein production". A dietary factor found in liver and yeast and in folic acid (*Ruegamer* and his collaborators) seems also necessary for optimal plasma protein production. *Robscheit-Robbins, Miller* and *Whipple* have measured the maximum haemoglobin and plasma protein production under the influence of amino acids (glycine, leucine, tryptophane, lysine, etc.).

On the other hand, *Orten's* observations do not seem to show that the addition of the amino acids essential for growth increases the haemoglobin formation.

Another factor which seems to play a certain part in the regulation of erythropoiesis is copper, whose absence can produce a certain anaemia. The importance of the supply of copper in the utilisation of iron for haemoglobin production has been demonstrated by *Hart* and *Elvehjiem* and more recently by *Schultze*. This author has demonstrated that copper is essential for the maintenance of maximum activity of the cytochrome oxidase of bone-marrow and for the production of haemoglobin. Copper can often cause a moderate increase of haemoglobin in anaemia due to blood loss (*Robscheit-Robbins* and *Whipple*).

The following case of secondary anaemia resisting iron treatment in a patient suffering from chronic gastro-enteritis, and who had during several months an insufficient diet, illustrates the favourable effect of amino acids on the anaemia.

Before the treatment: Hb., 49%; erythrocytes, 4,400,000; serum iron, 87 γ%.

After a treatment of one month with oral and parenteral iron: Hb., 46%; erythrocytes, 3,800,000; serum iron, 124 γ%.

After treatment with parenteral iron and injections of tryptophane, histidine, leucine for two weeks: Hb., 60%; erythrocytes, 4,340,000; serum iron, 72 γ%.

Finally, it is interesting to quote here the four cases of aplastic

anaemia described by *Cartwright, Wintrobe* and their collaborators; all these cases show an important increase of non-haemoglobin iron (222–318γ%).

Certain infections can permanently influence erythropoiesis without this being accompanied by a deficiency either of iron or amino acids. In the case of hypochromic anaemia based on syphilis the favourable effect of specific medication, in the face of failure of other methods of treatment, is well known. Certain forms of intoxication may also impede erythropoiesis; a proof of this is shown by the obstinate hypochromic anaemias which sometimes occur as the result of administration of Salvarsan preparations. Here is a typical example:

D. L., aged 49. Good general health, robust constitution. Menstruation from the age of 15, regular, rather abundant. No miscarriages; two normal pregnancies. At 44 menstruation was abnormally heavy for a few months. The following year cystitis, followed by a gradual deterioration of general condition. Removal of Fallopian tubes. At 48 casual discovery of a positive WR. After a first specific treatment the haemoglobin slowly fell to 62% and the number of red blood cells to 3.46 mill. (August 30). As the WR in the cerebro-spinal fluid still remained positive the patient again underwent one Salvarson and two bismuth treatments. Pale and weak. Family anamnesis: Father and mother died at an advanced age of pneumonia. A brother and four sisters in good health, three brothers died at an early age, one brother at 49 of leukemia, a sister at 39 also of leukemia.

On examination: Moderately good general condition. Weight 55.4 kg. Height 172 cm. Unusual pallor. Tongue clear, moist, not atrophic. Nasopharynx normal; heart and lungs normal. Non-histamine-resistant achlorhydria. Liver at the rib margin slightly painful upon palpation. The spleen extended beyond the rib margin by one finger's breadth; painful to pressure. Left Fallopian tube removed: extremities normal; urine—traces of protein, increased urobilinogen, Fehling dirty green sediment; a few leucocytes and epithelia.

	Hb. %	Erythrocytes Mill.	Reticulocytes %	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Oct. 18	38	3.16	2	15	104	0.61
	Rp. Mixtura pepsini and Campolon.					
	After 4 weeks of slight metrorrhagias:					
Dec. 2	38	3.30	14	34	71	
Dec. 9	38	3.30	2	14	45	0.71
	Rp. ferri reducti + mixtura pepsini + CeFerro intraven.					
Jan. 6	46	4.21		11	108	0.81
	Rp. combined treatment with iron per os, HCl, liver extracts and Vitamin B_2 and C parenterally.					
Apr. 17	58	4.00		15	93	0.70

On October 22nd a normal serum iron content is evidenced by an elevated serum iron curve following upon intravenous iron

administration, which only attained the initial values after three hours.

6 mg. iron intravenously.

0	10 minutes	1 hour	3 hours
98 γ%	150 γ%	143 γ%	144 γ%

The organism appeared to need no iron, probably owing to the inactivity of the bone-marrow. Actually the same curve showed quite a different shape on December 20th, at the end of a period of iron deficiency after repeated severe haemorrhages which were followed by increased bone-marrow activity:

0	10 minutes	1 hour	3 hours
86 γ%	117 γ%	97 γ%	84 γ%

The presence of serious hypochromic anaemia with a normal serum iron content recalls the normal serum iron content noted by *Heilmeyer* in panmyelophthisis. It is not the lack of iron that paralyses haematopoiesis, but inertia of the bone-marrow, the origin of which appears to be mainly functional. But the possibility is not excluded that in this case the unfavourable effect of the Salvarsan was based on a constitutional inferiority of the bone-marrow, recognised by the occurrence in the same family of two cases of leukaemia, which favoured the development of the hypochromic anaemia unaccompanied by a decreased serum iron content.

Protracted iron deficiency may eventually paralyse the ability of the bone-marrow to react to blood regeneration. It can exert the same effect on the bone-marrow as does a chronic infection, by rendering this organ incapable of fulfilling its functions despite the presence of sufficient quantities of all the substances needed for haemoglobin synthesis. Here is an example:

B. E., aged 45. Good health until her 39th year, when she began to suffer from heart-burn and repeated menorrhagia. Deterioration of general condition. Appetite moderate. Family anamnesis showed no indication of heart disease. On examination: Weight 33 kg., height 142 cm. Pale and slender, bad general condition. Tongue clear, moist, not atrophic. Hypochlorhydria. Spleen and liver normal. Faulty digestion of fats and starch. Lungs and heart normal. Myoma of uterus with menorrhagia.

	Hb. %	Erythrocytes Mill.	Reticulocytes %	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%	Weight kg.
Nov. 10	40	3.23	3	7	130	0.89	33.000
	Rp. Mixtura pepsini + Campolon.						
Nov. 30	41	4.15	5	10	94	1.12	33.000
	Combined treatment with liver extracts, iron, copper and manganese.						
Oct. 5	43	4.09	11	Traces	70	0.72	33.500
Nov. 2	58	4.39	15	26	86		36.000
Dec. 6	70	4.68	5	17	99		37.000

The repeated haemorrhage finally produced hyporegenerative anaemia. Campolon given; then better results attained by a combined treatment of liver extracts—iron, copper, manganese—which resulted in a slight haematopoietic reaction with decline of serum iron content. It is obvious that the determining factor of this anaemia was not an iron deficiency. The serum iron content, prior to each treatment, exceeded normal, falling during the bone-marrow regeneration to values only slightly below normal.

Finally we mention a case of panmyelophthisis, following upon a severe throat infection.

Mrs. D. B., aged 43. Blood examination: Haemoglobin 38%, erythrocytes 1,020,000, leucocytes 1200, neutroph. 22%, monoc. 10%, lymphocytes 68%, a few metamyelocytes. Serum bilirubin 0.15 mg.%, reticulocytes 1%.

A-iron = 0
B-iron = 130 γ%
C-iron = 75 γ%
D-iron = 45 γ%

Total iron = 250 γ%

In spite of the severe anaemia the serum iron showed normal values.

We thus had repeated opportunities to observe hypochromic anaemia without lowered serum iron content or any increased haemolysis. These anaemias were refractory to iron treatment. In one case the arrest of the haemoglobin formation appeared to be due to a deficiency of absorption of certain amino acids; in other cases to a certain functional inertia of the bone-marrow. These hypochromic anaemias without decreased serum iron content show, after intravenous injection of iron, that there exists with a normal serum iron content, or one approximating to normal, and a high and slowly declining serum iron curve.

6 mg. iron intravenously	0	10 minutes	1 hour	3 hours
A. E.	90 γ%	190 γ%	144 γ%	133 γ%
D. L.	98 γ%	150 γ%	143 γ%	144 γ%
J. R.	78 γ%	138 γ%	100 γ%	139 γ%

As a comparison here is a curve of a case of essential hypochromic anaemia:

E. M.	67 γ%	114 γ%	105 γ%	66 γ%

and that of a case of haemorrhagic anaemia:

68 γ%	110 γ%	70 γ%	70 γ%

(*b*) *Haemolytic Anaemias*

In this group of anaemias excessive haemolysis destroys the balance between the destruction and the regeneration of the erythrocytes. We have seen above the effects of haemolysis on iron metabolism. Haemoglobin is transferred to the plasma, followed by conversion into bilirubin, partly by way of pseudo-haemoglobin formation, and the release of iron. The decomposition occurs partly in the reticulo-endothelial system, partly in the circulating blood, according to the mechanism described by *Barkan*.

Acute haemolysis quickly releases A-, B- and possibly even C-iron. A-iron hardly diffuses in the serum, whilst the iron which is closely bound in complexes passes quickly into the plasma. The slow haemolysis only slightly influences the serum iron, as apparently the organism has sufficient time in which to retain, and after that to decompose and eliminate, the products of haemoglobin decomposition.

The aetiology of the haemolytic anaemias is manifold. Apart from the constitutional familial haemolysis, we are acquainted with a toxic form (arsenic, carbolic acid, phenylhydrazine, nitrobenzole, etc.) and a toxic-infectious form (infection from B. perfringens, certain types of streptococci, malaria, etc.). The most powerful reactions of blood regeneration after haemolysis are found in haemolytic icterus. In that condition none of the haemolysing factors impede the bone-marrow activity at the same time, as is often the case in toxic or toxic-infectious haemolysis. In acute haemolysis the products of blood pigment decomposition which are suddenly liberated in great quantities are utilised for blood formation. So we see that the end of a severe haemolytic attack is characterised, on the one hand, by an increase of the reticulocytes in the blood, and on the other hand, by the sudden deep decline of the serum iron values, which prior to the attack had attained very high values. Not all the iron liberated by haemolysis can be utilised for the resynthesis of haemoglobin; one part, as we shall see, is eliminated in the bile, as long as the serum iron content is high. Thus, in the case of one of our patients, during slight haemolysis the value of the iron in the bile rose from 100 γ% to 250 γ%. We are not giving any clinical examples at this place as in the section on Haemolysis (on pages 80–91) we have discussed at length various cases of haemolysis and haemolytic anaemia.

(*c*) *Pernicious Anaemia*

Pernicious anaemia is characterised by a very deep-rooted disturbance of erythropoiesis, in which the bone-marrow builds a

quantity of red blood cells of inferior functional capacity. This inferiority of the erythrocytes is indicated, among other symptoms, by increased haemolysis. Hence it is to be expected that pernicious anaemia will present similar serum iron conditions as are found in haemolysis. But that is only partly the case, as the pathogenesis of this disease is much more complicated than a condition of merely increased blood destruction. Actually it represents a severe disturbance of erythrocyte formation, equivalent to a state of reversion to the embryonal blood picture—a condition, as we shall see later, which involves a fundamental change of the iron and pigment metabolism.

The rapid return to a normal mechanism of regeneration in the bone-marrow in pernicious anaemia enables us to obtain a certain insight into the processes of blood regeneration. We give a few examples of this:

L. J., aged 40.

	Hb. %	Erythrocytes Mill.	Reticulocytes %	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Dec. 10	45	1.78	6	110	181	1.25
	Rp. Campolon.					
Dec. 17	45	1.94	30	84	108	1.36
Dec. 23	46	2.30	39	10	62	0.69
Jan. 20	57	3.07	3	20	50	0.59
	Rp. Ferri reducti + diluted HCl per os.					
Feb. 7	68	3.99	1	10	50	0.31
Mar. 7	65	3.94	1	20	70	0.60
	Rp. Campolon + ferri reducti and Acidol-Pepsin.					
May 10	70	5.3	1	Traces	25	0.25

If now we compare the values of the lightly bound iron (*Barkan*) with that of the closely bound iron (*Heilmeyer*) and with the serum-bilirubin content, we reach in this case the following results (in which the closely bound iron represents the difference between the *Heilmeyer* iron and the *Barkan* iron):

	Barkan iron	B-iron	Bilirubin
December 10 ..	110 γ%	71 γ%	1.25 mg.%
„ 17 ..	84 γ%	24 γ%	1.36 mg.%
„ 23 ..	10 γ%	52 γ%	0.96 mg.%
January 20 ..	20 γ%	30 γ%	0.59 mg.%
February 7 ..	10 γ%	40 γ%	0.31 mg.%
March 7 ..	20 γ%	50 γ%	0.60 mg.%
May 10 ..	Traces	25 γ%	0.25 mg.%

If we compare these two fractions with the bilirubin content we immediately see that the value of the *Barkan* iron is to a certain extent parallel with the bilirubin level, although this does not apply to the values of the closely bound iron. This circumstance coincides with our findings in connection with haemolysis.

The treatment with extracts of liver soon re-established a normal ripening of the red blood cells and corrected the pathological haemolysis. Thus both the serum bilirubin content and the serum iron content decreased. As soon as blood regeneration began, as evidenced by the reticulocyte crisis (of 39%), we witnessed a sudden decrease of the " easily split-off iron ", whilst the more closely bound fraction tended rather to increase. The organism therefore appeared to utilise the " easily split-off iron " primarily for the blood regeneration; that is to say, the iron which was only lightly bound in complexes.

It should be remarked in this connection that the colour index very rapidly fell to values around 1, where it remained for a long time. This fact is explained by a prolonged marked reduction of the serum iron, a reduction which continued, even when the bone-marrow regeneration had ceased to be so intensive. In this case there was a deficiency of iron, probably due to insufficient intestinal absorption of iron. Only after five months of combined treatment *per os* with liver extract and iron was the anaemia cured But there still remained a reduction of the serum iron, proof of which is found in the values of 25 γ% of *Heilmeyer* serum iron and in the traces of *Barkan* iron.

It might be considered remarkable that in pernicious anaemia a condition of iron deficiency should develop at all. But this is obvious from the clinical observations, which show that in certain cases liver treatment is followed by a secondary anaemia because the iron reserves of the organism have been used up during the phase of regeneration following upon the liver treatment. In these rare instances the hyperchromic character of the original anaemia may reappear if the patient is treated with iron. We know that in pernicious anaemia the iron reserves are great, as a result of the increased destruction of blood, as is shown by the excess of iron contained in the tissues in this form of anaemia. But we have seen that only a certain kind of iron, above all fractions A and B, is needed for blood formation; the rest of the metal, representing most of the iron-containing pigments of the tissues, is unable to take part in blood regeneration.

The fractional determination of the serum iron enables us to approach more closely to the problem of the conversions of the metal in erythropoiesis:

(1) H. C. (woman), aged 36.

	Hb.	Erythro-cytes	Reticulo-cytes	Bili-rubin	Serum Iron: Aqueous extract: Direct determination	Serum Iron: Aqueous extract: Incineration of extract	Serum Iron: 6N HCl extract: Direct determination	Serum Iron: 6N HCl extract: Incineration of extract	Total: Incineration of extract
	%	Mill.	%	mg.%	γ%	γ%	γ%	γ%	%
May 2	48	1.61	0	1.4	105	460	450	485	495
May 13	Rp. Liver extract.								
May 13	56	2.77	56	2.5	25	145	140	185	200
May 21	62	3.55	11	0.5	30	180	230	240	305
May 30	68	3.68	10	0.4	40	240	160	290	405
June 18	72	3.86	1	0.05	10	170	130	250	350

In this case we again find increased serum iron and blood bilirubin content; this was the result of haemolysis which rapidly receded under the influence of the treatment. From the results of the analyses we are able to calculate the various fractions:

	Before treatment	Liver extract treatment			
	May 2	May 13	May 21	May 30	June 18
A-iron γ% ..	105	25	30	40	5
B-iron γ% ..	345	115	200	120	125
C-iron γ% ..	35	45	10	130	120
D-iron γ% ..	10	15	65	115	100
Total iron γ% ..	495	210	305	405	350

Under the influence of the treatment the total iron rapidly fell to 2/5 of the initial value, rising again, once the anaemic condition was nearly cleared up, to 4/5 of this same value. But then the iron was in a quite different form. Before the treatment the fractions A and B, i.e. the separable iron, represented 9/10 of the non-haemoglobin iron, and as shown by the incineration of our " aqueous extract ", 460 γ% out of a total quantity of 495 γ% was soluble in trichlor-acetic acid. Under the influence of liver extract these fractions diminished, to make way for an iron which was more closely bound in complexes and which was chiefly made up of the non-separable complexes and of iron of the protein precipitate. Therefore, in the course of treatment these fractions passed from 35 γ% to 130 γ% and from 10 γ% to 115 γ%. It should further be remarked that after treatment with liver extract only

half of the non-haemoglobin serum iron was still soluble in the trichlor-acetic acid. The liver extract treatment thus completely reconverted the serum iron, both qualitatively and quantitatively. At the time of blood regeneration we noted a considerable reduction of the serum iron, a reduction that chiefly affected the iron fractions, which are in the form of " easily split-off " complexes. Gradually the total amount of serum iron rose again to nearly its initial values, owing to a progressive increase of those iron fractions which were more closely or irreversibly bound to complexes and which could not be estimated according to the ordinary methods of serum iron determination.

A second observation of a mild case of pernicious anaemia in an elderly person in whose case the blood regeneration proceeded in a less intensive manner led to the same conclusions.

(2) T. J., aged 60 (see Diagram 6).

					Serum Iron				
					Aqueous extract		6N HCl extract		Total
	Hb.	Erythro-cytes	Reticulo-cytes	Bili-rubin	Direct determin-ation	Incinera-tion of extract	Direct determin-ation	Incinera-tion of extract	Incinera-tion of extract
	%	Mill.	%	mg.%	γ%	γ%	γ%	γ%	γ%
Apr. 24	57	2.1	0	2.1	75	130	220	290	310
	Rp. liver extract.								
May 1	63	2.45	24	0.8	8	37	140	180	218
May 19	75	3.85	0	0.5	15	220	125	230	340
May 30	81	3.8	3	0.5	5	205	115	235	335
June 16	83	4.25	5	0.25	0		120	180	280

The calculation gives us the values of the 4 different fractions as follows:

	Before treatment	Treatment with Liver Extract			
	April 24	May 1	May 19	May 30	June 16
A-iron γ% ..	75	8	15	5	0
B-iron γ% ..	145	132	110	110	120
C-iron γ% ..	70	40	105	120	60
D-iron γ% ..	20	38	110	100	100
Total iron ..	310	218	340	335	280

We again noted before treatment a very pronounced preponderance of the separable complexes, then, under the influence of the treatment, a progressive increase of the fractions of the non-separable complexes and of the iron of the protein precipitate. The decline of the " easily split-off " fraction was not so marked as in the case of the previous patient, and this circumstance can

probably be correlated with a less active haematopoietic reaction in this particular case. An interesting fact is that in this patient the fraction of the B-iron was also but slightly involved in the resumption of erythropoiesis. This different behaviour of the B-iron in these two cases appears to be connected with the less active erythropoiesis of the second case. Indeed, whenever the treatment provoked an active resumption of erythropoiesis a very pronounced mobilisation of iron could be observed, either of the lightly bound or the firmly bound fractions. If the reaction of the blood formation was less intensive what we chiefly saw was a mobilisation of the A-iron, which was lightly bound to the complexes. The firmly bound iron decreased only slightly in the serum. Finally, it should be remarked that after liver extract treatment the total iron was on a higher level than before the treatment, as the increase of the iron fractions C and D, i.e. the non-separable iron, was greater, due to the influence of the liver extracts, than the corresponding decrease of the " easily split-off " iron forms.

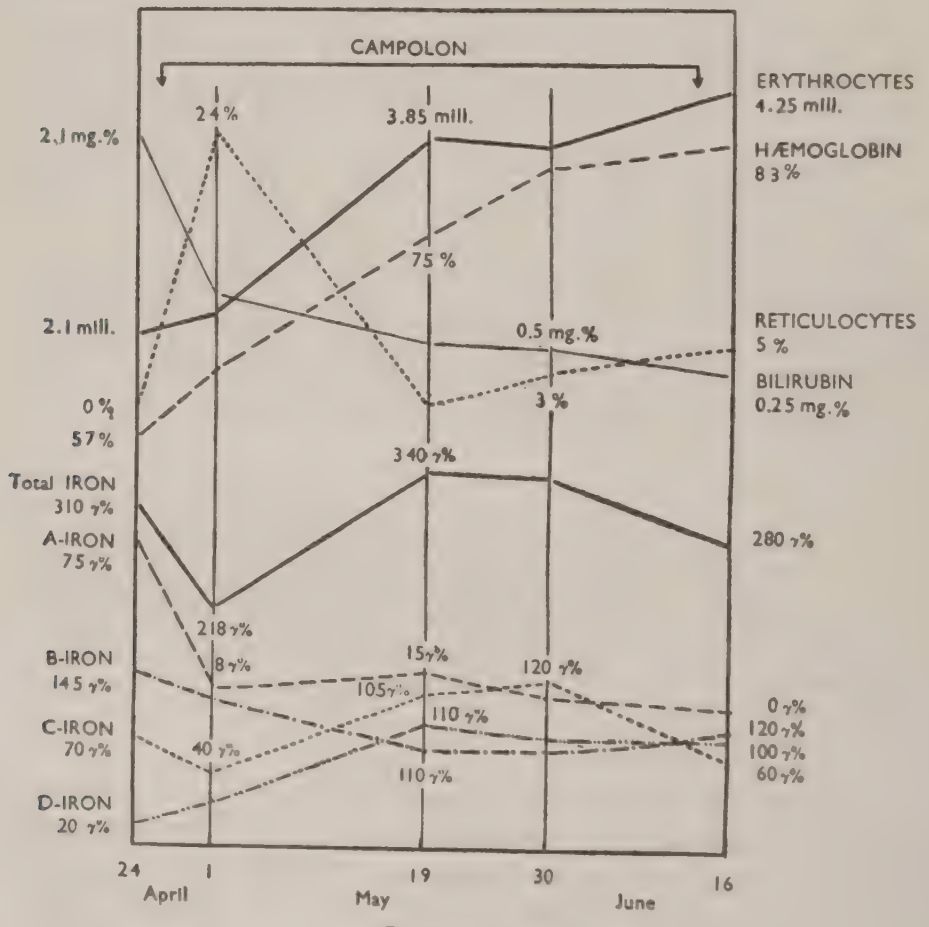

DIAGRAM 6
Serum iron fluctuations in the course of pernicious anaemia during liver treatment.

We add two further observations which show that the change of the serum iron level as a result of the effect of blood regeneration after liver treatment could be clearly seen.

G. C., aged 70. *Pernicious anaemia.*
Blood picture before treatment:

Hb.	Erythrocytes	Reticulocytes	Bilirubinaemia		
50%	2.12 Mill.	4%	1.45 mg.%	A-iron	5 γ%
				B-iron	295 γ%
				C-iron	80 γ%
				D-iron	10 γ%
				Total iron	360 γ%

Ten days after start of treatment with liver extract:

Hb.	Erythrocytes	Reticulocytes	Bilirubinaemia		
62%	3.19 Mill.	43%	0.85 mg.%	A-iron	0 γ%
				B-iron	120 γ%
				C-iron	70 γ%
				D-iron	10 γ%
				Total iron	200 γ%

T. M. A., aged 58. Non-megalocytous, hyperchromic anaemia, reacting well to liver extracts.

					Serum Iron				
					Aqueous extract		6N HCl extract		Total
	Hb.	Erythrocytes	Reticulocytes	Bilirubin	Direct determination	Incineration of extract	Direct determination	Incineration of extract	Incineration of extract
	%	Mill.	%	mg.%	γ%	γ%	γ%	γ%	γ%
Apr. 15	67	3.090	5	0.05	30		195	220	260
	Rp. liver extract.								
May 19	87	5.120	10	0.20	8		105	203	285

In this case the total iron also slightly increased as a result of the influence of the liver extract treatment, but the conversions produced by the treatment affected the form of the iron much more than its total serum content.

In anaemias of the pernicious type the serum iron and particularly the separable iron is exceedingly high. In pernicious anaemia nearly all the non-haemoglobin iron in the serum is present in the separable form, and the non-separable complex and the iron of the protein precipitate represent only a very small part, sometimes hardly $\frac{1}{10}$ of the total serum iron. This condition appears to be partly related to the lowered protein content of the blood, so frequently found in pernicious anaemia.

The question now arises whether the non-separable iron com-

plexes represent simple final products of iron metabolism, that is, residual constituents which can no longer be utilised and are ready for excretion, or whether they are, in part at least, the products of the conversion of the metal which is taking place in the organism and which is needed to serve other purposes (especially in the bone-marrow). Although various facts indicate that the latter hypothesis may be correct, it would suffice merely to mention the constant presence of a great number of these fractions in normal blood formation.

The state of the non-haemoglobin iron and its fractions in pernicious anaemia is strikingly similar, as we shall see, to that which we have found in certain hepatic diseases. Moreover, we are tempted to assume in pernicious anaemia an alteration of the iron metabolism, not only as the consequence of the increased haemolysis and of the pathological erythrocyte formation, but also due to the involvement of the liver and reticulum in the pathogenesis of this form of anaemia.

The increase of A- and B-irons, which partly correspond to the fractions of *Barkan* and *Heilmeyer*, recalls our observations in connection with haemolysis. But the analysis of the two other fractions, C and D, places the problem in quite a different light. Actually in experimental haemolysis the C-iron decreases in the acute form, both *in vitro* and *in vivo*, whilst the D-iron increases in acute and chronic haemolysis. This last phenomenon, although often temporary, is doubtless to be attributed to an enrichment of the serum, prior to the destruction of the red blood cells, in haemoglobin and substances needed for erythrocyte formation (globin, protein from the stroma of the erythrocytes, etc.). In pernicious anaemia, on the other hand, there is a conspicuous reduction of the irons C and D; for, despite a pronounced increase of irons A and B, the total serum iron often remains normal. The reduction of the non-separable iron (C and D) is partly due to the lowered blood protein content in pernicious anaemia, and thereby it differentiates the iron metabolism of simple haemolysis from that of pernicious anaemia.

The treatment with liver extracts, which effects prompt regeneration of the bone-marrow, very soon changes the picture of the distribution of the serum iron. Indeed, the value of the A- and B-irons very quickly falls as a result of the powerful stimulation of the blood regeneration—as soon as the reticulocyte crisis starts and the erythrocytopenia begins to recede. During this phase it is even possible to observe a sudden reduction of the serum iron content (see also *Lederer*). Only a few weeks later, when the anaemia indicates a distinct improvement and the values of the A- and B-irons show a tendency towards stabilisation, do the fractions C and

D slowly rise. Hence it must be assumed that the erythropoietic regeneration is based principally on the participation of irons A and B. The two other fractions depend partly upon the protein content of the serum, especially the D-iron; but in part also upon the further utilisation of the separable iron during erythropoiesis. Accordingly they only return to normal values after the A and B fractions have already been mobilised for a long time.

Finally, the distinct increase of the separable iron is not only a manifestation of increased haemolysis, but also of an insufficient consumption of the metal for the formation of haemoglobin. For we know from the investigations of *Borst* and *Königsdörfer, Vannotti, Waldenström* and others, that in pernicious anaemia the bone-marrow shows a clear increase of porphyrin formation, i.e. of an iron-free blood pigment. Hence we must assume that the erythroblast is incapable, as in early embryonal life, of producing a complete haemoglobin synthesis from porphyrin and iron. It can, therefore, only use the proffered iron to a limited extent.

Using radioactive iron, *Hahn, Sheppard* and *Caruthers* were able to see that there is no difference in the absorption of iron and its utilisation under the influence of liver extracts in the normal subject. In two cases of pernicious anaemia, *Dubach, Moore* and *Minnich* were able to see that, before treatment, only 20–40% of the radioactive iron injected intravenously was utilised for the formation of the haemoglobin. On the other hand, the reticulocyte crisis which is provoked by liver extract causes a sharp increase in circulating radioactive iron and within 2–3 weeks all of the iron was synthesised into haemoglobin.

Finally, we were able to observe the same variations of non-haemoglobin iron in cases of pernicious anaemia treated with folic acid. In relation to the weaker response of the organism to treatment with folic acid, we often see a less rapid and less marked fall of circulating iron after the reticulocyte crisis.

		Folic acid					
Date: June	5	7	9	11	13	15	17
Hb.%	43	38	39	45	56	60	62
Red cells (mill.)	1.85	1.34	1.42	1.65	2.33	2.35	2.4
Reticulocytes %	8	8	36	316	133	67	39
Non-haemoglobin iron γ%	255		250		80		55

Analogous observations were recently described by *Cartwright, Wintrobe* and his collaborators.

In the cases of non-pernicious macrocytic hyperchromic

anaemia, i.e. in the rather rare forms of pseudo-pernicious anaemia observed with hypovitaminosis of the B complex and in the pseudo-pernicious "Wills's anaemia" (tropical anaemia), our analysis showed generally a normal percentage of non-haemoglobin iron. In fact, there is no intense hemolysis in these cases and the reason for the anaemia is to be found in the disturbances of intestinal absorption of the anti-anaemic principle or of important factors in the regulation of the erythropoiesis, such as certain vitamins of the B group.

II. Iron and the Reticulo-endothelial System

We have just seen the significance of erythropoiesis and haemolysis in connection with the distribution of the various serum iron fractions. Since the reticulo-endothelial system is known to be functionally closely connected with the bone-marrow in the destruction of the red blood cells, we deem it indispensable to follow up the problem of non-haemoglobin iron metabolism within the domain of reticular activity.

Before doing so we will give some data selected from the literature. *Thoenes* and *Aschaffenburg* mention a lowering of the serum iron in injury of the reticulum after diphtheritic poisoning or as the result of the action of colloidal metal solutions. In the latter case these authors noted a decrease of the iron content immediately after the obstructive injection. This decrease was transient and disappeared after forty-eight hours. *Barkan* also observed this phenomenon, but when the reticulum was blocked by repeated injections these perceptible fluctuations could no longer be noted.

It would therefore appear that the reticulo-endothelium takes an active part in iron metabolism. In order to obtain more precise data regarding this point we undertook a series of experiments on rabbits whose reticulo-endothelial system had been more or less completely blocked by various methods.

A first series of experiments was designed to check the accuracy of the observations of *Thoenes* and *Aschaffenburg* relative to a temporary reduction of the *Heilmeyer* serum iron and of the *Barkan* iron in the blood, following upon an obstructive injection.

If the injections are repeated at intervals of three to four days it can be seen that after fifteen days there is a marked reduction in the number of red blood corpuscles and the haemoglobin, accompanied by a corresponding decrease of iron. If the injections are regularly continued, a slow but definite rise of the haemoglobin content is noted and the erythrocytes are still slower in increasing. During this stage the serum iron content shows a considerable rise. The influence of the colloidal metals on the reticulum shows two phases: the first is characterised by the typical obstruction of the blocked reticulum. Then, after a certain number of injections have been given, the reticulo-endothelial system reacts by increased activity, which is histologically revealed by the hypertrophy of many of its cells. The obstruction which first prevailed is therefore followed by a stimulation which explains the second phase with its succeeding rise in serum iron as the manifestation of increased haemolysis. This mobilisation of the iron soon appears to exert an influence on the blood (increase of haemoglobin

and of the number of erythrocytes); but of course all these processes are dependent upon various factors, such as the degree of obstruction, the strength of the reticular stimulus, and particularly upon the relationship existing between the activity of the reticulum in the liver and spleen and that existing in the bone-marrow.

As a matter of fact, the reticulo-endothelial system does not react in the same way in all the organs to the injection of obstructive substances. The reticulum of the bone-marrow appears to be slower in establishing contact with the injected substances than are the spleen and liver, probably because the reticulum of these organs takes up most of these substances. *Kadruka* was able by means of X-rays to follow the slow passage of injected thorotrast from the depots of the liver to the bone-marrow. This fact is of practical importance for the interpretation of the dependence of iron metabolism upon the functioning of the reticulum of the bone-marrow. For, at the moment that the reticulo-endothelium of the liver and spleen registers a stimulus as a result of repeated injections of obstructive substances, the reticulum of the bone-marrow is still in the first phase of obstruction by blocking.

In order to completely eliminate the bone-marrow we repeated our obstructive injections after splenectomy. The results differed from those obtained in our first experiments as follows: The iron curve rose regularly for a certain length of time, whilst the erythrocytes and haemoglobin remained at the same level. If the blocking was undertaken at great intervals of time, the erythrocytes and haemoglobin usually increased; but if the injections were repeated at more rapid intervals, a very severe condition of anaemia quickly set in. In both cases the iron diminished more or less slowly. The blocking of the bone-marrow paralysed its activity; after a preliminary period the number of red blood cells decreased, without there being any perceptible changes. During the first phase the iron increased, it ceased to be consumed by the bone-marrow, which was now blocked, and as long as resorption proceeded regularly the iron continued to remain in circulation for a long time, as it could no longer become deposited in the reticulum. During this phase, according to the observations of *Lauro*, an increase of iron elimination could be noted. If the blockage was strengthened, a rapid diminuation of the blood values and considerable obstruction of the iron circulation could be noted; if, on the other hand, the reticulum received a stimulus under the influence of the injections, the erythropoiesis tended rather to be strengthened. The erythrocytes increased in number and the consumption of the circulating iron was also augmented.

These observations, as well as others which relate to the functioning of the bone-marrow in lead poisoning (see pp. 152–6)

clearly show the importance of the role of the reticulum in the regulation both of the non-haemoglobin iron and of the blood-forming function of the bone-marrow. If the reticulum of the bone-marrow is paralysed the erythroblast loses the power of utilising the non-haemoglobin iron, and this is followed by anaemia. The obstruction of the spleno-hepatic reticulum is accompanied by a decrease of physiological haemolysis and iron deposition in the organs of storage. The lack of non-haemoglobin iron results in a suspension of haemoglobin formation. It might be assumed that the erythroblast would possibly produce an iron-free blood pigment, i.e. a porphyrin, but such is not the case. Despite the most systematic of investigations, we have never been able to discover the existence of increased porphyrin formation, even after the bone-marrow has been completely blocked.

Therefore, the reticulum of the bone-marrow possesses a definite regulatory significance in the metabolism, not only of iron, but also of the chromogens (porphyrin ring) of the blood. Its non-participation usually leads to a pronounced inhibition of haemoglobin formation in the erythroblasts.

We thus see that the reticulo-endothelial system plays an important part in haematopoiesis, namely, in the double sense of receiver and possibly also of an organ of deposit for the basic substances needed for haemoglobin formation (iron and chromogen), and as a conveyor to transmit these products to the erythroblasts for synthesis. The reticulum shows certain functional differences, depending upon whether it is situated at the periphery of the erythropoietic system (spleen and liver) or in its centre (bone-marrow). The stoppage is effected neither at the same time nor in the same manner, and the metabolic reactions of the non-haemoglobin iron are different to stimulation from what they are to obstruction. These functional differences in the reaction of the various organs are closely associated with the essential mechanism of blood formation; thus the reticulum of the bone-marrow would not possess the same function of blood destruction as would that of the organs of haemolysis (spleen and liver). On the other hand, it possesses a particular affinity for the fixation and deposition of non-haemoglobin iron and chromogens, which are needed for the synthesis of haemoglobin. Finally, it is the bone-marrow which supplies the erythroblasts with the products, taken from the circulation, which serve for haemoglobin synthesis.

In human pathology the various forms of obstruction and of bone-marrow destruction, i.e. the aplastic anaemias, show a certain analogy with the phenomena which we have just described. As we have already noted in the course of our study of the porphyrins, normal, sometimes even increased, non-haemoglobin iron values

are usually found in these cases. This determination is all the more interesting in view of the fact that the cases observed usually assume the form of very severe anaemia, nearly always of a hypochromic character. *Heilmeyer* and *Plötner* recorded five cases of myelophthisis, and we ourselves also mentioned, in the preceding chapter, such cases showing a normal serum iron content, that is, a very marked increase, as compared with the haemoglobin values. The value of the *Barkan* iron was also normal, in one case it was even definitely increased. These clinical observations confirm the experimental findings which have just been described. The most convincing conclusion to be deduced from them is that obstruction of the haematopoietical activity of the bone-marrow greatly influences the utilisation of the circulating non-haemoglobin iron which is being collected in the blood. This accumulation affects not only the iron fraction which cannot easily be separated, but also the easily split-off fraction, thus confirming our observations of blood regeneration in the course of pernicious anaemia.

In yet another case of severe anaemia, caused by extensive carcinoma-metastases in the bone-marrow, we found normal values for the circulating iron. The destruction of the marrow tissues had started the anaemia and had thus prevented the organism from utilising its iron for haemoglobin formation.

The function of the reticulo-endothelial system as an iron depot in the organism can be seen in the reactivated increase of the serum iron content in human subjects twenty-four hours after an acute haemorrhage of 300–500 cc. This fact, to which attention has already been drawn by *Heilmeyer* and *Vannotti*, was mentioned above during the discussion of haemorrhagic anaemia.

Moreover, iron metabolism is also seen to possess interesting relationships with the reticulo-endothelial system under conditions of infection. In the course of acute or chronic infections the serum iron content diminishes, sometimes even falling to extremely low values, without the occurrence of any associated diminution of physiological haemolysis. *Thoenes* and *Aschaffen-*

Day of disease	Temp.	Blood reduction	Hb.	Erythrocytes	Leucocytes	Serum Iron	
						A-iron	B-iron
	°C.	mm.	%	Mill.		γ%	γ%
3	38.5	40	80	5.5	9100	Traces	42
8	37.5	67	80	5	12000	„	20
15	37.3	54	80	4.9	9000	„	30
40	36.8	15	80	4.9	5400	20	210

burg, Heilmeyer and *Plötner, Heilmeyer, Keiderling* and *Stüwe, Hemmeler, Büchmann* and *Heyl,* and we ourselves have frequently stressed this point.

The table gives a typical instance of this fall in serum iron in a 33-year-old man with acute broncho-pneumonia (of the lower pulmonary lobe).

In the acute infection with high fever and increased metabolism a reduction of the serum iron content could partly be accounted for by an augmented consumption of the iron as a catalyst of the cell oxidation; but one of the main causes lies in the prophylactic reaction of the infected organism which, as shown by *Wallbach* and *Hettche,* as well as by *Heilmeyer, Schairer, Ehrich* and *Lange,* stores iron in the reticulo-endothelial system. On the other hand, the same reticulo-endothelial elements which are distributed throughout the organism, particularly in the spleen and liver, participate in the neutralisation of the toxic bacteriological products. Hence we are led to assume that iron affects the toxins indirectly via the reticulum.

According to *Heubner* iron storage in the reticulum might in infectious diseases indicate something other than the potency of the prophylactic processes of this system. According to this author there is increased reception of circulating iron by the reticulum, which as a result of the infection is in a state of augmented activity.

The observations of *Hettche* regarding experimental diphtheritic intoxication show that oral iron administration has no effect on the length of life of the experimental animal, but that it is considerably extended by parenteral iron administration. In these animals the iron deposited in the spleen, if administered *per os,* did not seem to fluctuate, whilst in the animal treated by injections it appeared to increase very much. The author therefore distinguishes a " stimulating iron " which develops a prophylactic effect towards the toxin, from an inactive iron which is absorbed from the intestine. It is still undecided whether the iron introduced *per os* is really absorbed; at all events it can be assumed that the metal possesses and exerts an antitoxic effect in diphtheria.

Heilmeyer, Keiderling and *Stüwe* have fairly regularly observed the co-existence of a reduction of iron and an increase of the serum copper in the course of infections or of toxic conditions. These authors interpret this phenomenon as the expression of a prophylactic reaction on the part of the organism to the infection.

Wohlfeil, who followed the development of the diphtheric infection under the influence of heavy metals, notes that in the localised processes the iron collects almost exclusively at the focus

of infection, particularly where there is most extensive toxin formation. In his opinion the iron either exerts a paralysing effect on the toxins or activates certain processes of immunisation. In this connection it may be well to recall the activating effect of iron on vitamin C, as well as the inhibition which many heavy metals exercise on certain enzymes. If the organism's power of resistance fails the serum iron content may rise; before death occurs it may attain normal values, indicating a definite elimination of the reticular activity, the functional condition of which determines the immunity. (The curves of the serum iron under the influence of fever will be given later in the section entitled " Iron and General Metabolic Disturbances ", p. 195.)

Among the numerous infectious-inflammatory diseases in which iron metabolism has already been extensively studied we have directed special attention to tuberculosis. In this disease, which assumes a chronic course interrupted by exacerbations, the factors of temperature, weight, blood counts, rapidity of blood sedimentation, and the tuberculin reaction, furnish us with fairly reliable data regarding the state of resistance and the specific allergy of the organism, as well as indirect information relative to the prophylactic mechanisms which in part represent the function of the reticular activity.

Various authors have already taken up the question. *Heilmeyer* and *Plötner* have found with fair regularity a distinct diminution of the serum iron content, particularly in its progressive forms. These authors reached the conclusion that the acute tuberculous attacks are characterised by a reduction of the serum iron, even before there is any characteristic increase in the rapidity of the blood sedimentation. *Hirvonen* and *Schaefer* frequently observed a distinct reduction of the serum iron content in tuberculosis. *Vahlquist* described the same phenomena as occurring in tuberculous infection in children, whilst *Thoenes* and *Aschaffenburg* were unable to note any special changes. In addition to a reduced serum iron. *Heilmeyer, Keiderling* and *Stüwe* recently described a marked rise of the serum copper in progressive tuberculosis, and only a slight rise in the inactive form.

Our observations relate less to the statistical determination of the serum iron level in tuberculosis in general than to the iron fluctuations in those progressive attacks which are associated with the humoral reactions of the organism, and to a comparison of the iron curve with the clinical symptoms, such as temperature, blood sedimentation, blood counts, the Mantoux reaction (simultaneous tests of 0.1 cc. of a solution of tuberculin 1/1,000,000 ($=TbK^{-6}$) and 1/10,000 ($=TbK^{-4}$). The mechanism of haemolysis was assessed by the serum bilirubin.

Here are a few cases:

Forms with acute onset and rapid development.

1. W. F., aged 17. *Tuberclous primary infection.*

	Temp. °C	Pulse	Sedimentation rate mm.	−⁶ TbK −⁴ mm.	mm.	Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
Mar. 23	37·0	80	9	3	30	106	6·0	0·53	50	5
Apr. 5	37·0	80	11	9	30	99	6·0	1·02	12	5
The radiological shadow, which at first was extensive and uniform at the outset, next showed the typical picture of the primary complex.										
Apr. 20	37·0	76	6	4	24	92	5·9	0·74	72	25
Apr. 28	36·6	72	5	6	26	94	5·9	0·88	90	25
Very slow disappearance of the pulmonary shadows. The patient was sent to a sanatorium.										

2. S. I., aged 21, had successively scarlet fever and erythema exsudativum multiforme—1937 tuberculous lung infiltration on the right. Mantoux positive, B.K.=0. Prompt resolution. Resumption of work and good health. For the past 2 weeks (i.e. from February 10, 1940) *progressive cavernous pulmonary right tuberculosis.* Cough, sputum. B.K. positive. *Debility.*

	Temp. °C	Pulse	Sedimentation rate mm.	−⁶ TbK −⁴ mm.	mm.	Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
Mar. 23	37·7	92	30	5	30	90	4·4	0·68	60	5
Apr. 4	36·7	87	29	20	45	85	4·5	1·55	190	10
Treated in sanatorium, marked improvement. In October patient was well and had gained 5 kg.										
Oct. 2	36·5	84	25	0	25	74	4·2	0·48	80	10

3. Z. N., aged 25. *Fulminant, exudative, cavernous pulmonary tuberculosis of the right upper lobe. Debility.*

	Temp. °C	Pulse	Sedimentation rate mm.	−⁶ TbK −⁴ mm.	mm.	Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
Mar. 22	37·3	64	45	2	25	79	4·3	0·86	32	Traces
Mar. 28	37·3	87	48	—	—	76	4·1	0·56	20	15
Clinical deterioration.										

4. M. M., aged 35. *Chronic alcoholic, bilateral cavernous pulmonary tuberculosis with acute febrile attack one month previously.*

	Temp. °C	Pulse	Sedimentation rate mm.	−⁶ TbK −⁴ mm.	mm.	Hb. %	Erythrocytes mill.	Leukocytes	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
Apr. 3	38·0	160	47	3	13	88	4·4	7,700	0·92	15	10
Apr. 22	37·5	125	39	4	100*	88	4·4	12,900	0·68	62	15
During period of convalescence.											

*Extensive cutaneous infiltration. Reaction to 0·1 cc. TbK. 1/10,000 with no increase of temperature, nor focal reaction which could be clinically demonstrated. The patient was decidedly better.

Forms with slow development.

5. P. E., aged 25. *Chronic pulmonary tuberculosis of both apices.* No fever. B.K.+, with alternating deterioration and improvement of the general condition. The disease started 3 years before with acute attacks which receded. Good general condition. At time of examination, acute infiltration of right upper lobe.

	Temp. °C	Pulse	Sedimentation-rate mm.	−6 TbK −4 mm.		Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ %	Barka iron γ%
Mar. 23	37·5	84	17	0	35	92	4·5	1·50	70	15
Apr. 5	36·9	80	12	2	54	86	5·0	0·74	40	15
Retrogression of radiological signs.										
Apr. 21	36·8	59	10	0	12	87	5·4	1·03	148	30
May 3	36·8	60	11	0	0	85	5·0	0·81	160	30
The patient spent three months in a sanatorium, which he left against the wish of his physician. Increase i weight, normal temp. Cough and sputum—0 ; B.K.—0. But the tuberculosis was considered still active.										
Aug. 21	36·8	60	19	0	0	91	5·2	1·22	46	0
Aug. 29	36·8	76	21	0	0	91	4·9	0·91	95	8
Sep. 12	36·8	80	20	3	28	90	4·8	0·78	55	Trace
Sep. 26	36·4	85	24	—	—	91	4·8	0·48	56	10
Oct. 25	36·5	80	18	—	—	84	4·7	1·13	55	11

6. D. E., aged 31. *Bilateral tuberculosis of apices, slowly progressive.* B.K.=0. Onset 3 years before, acute, febrile. Came for examination on account of debility and deterioration of general condition.

	Temp. °C	Pulse	Sedimentation rate mm.	−6 TbK −4 mm.		Hb. %	Bilirubin mg.%	Heilmeyer iron γ %	Barka iron γ%
Mar. 11 ...	38·5	110	30	2	4	79	0·34	45	10
Apr. 13 ...	36·8	90	45	4	30	76	0·54	42	20
May 10 ...	36·5	90	37	0	30	76	0·59	45	12
June 8 ...	36·5	90	20	0	25	78	0·48	132	20
July 18 ...	36·4	84	15	0	20	72	0·39	179	10
Sept. 7 ...	36·4	80	11	—	—	81	0·56	87	14
Oct. 8 ...	36·4	80	9	3	7	71	0·98	96	20

7. R. J., aged 27. *Spondylitis.* Previous epididymectomy: *Renal tuberculosis.*

	Weight Kg.	Pulse	Temp. °C	Sedimentation rate mm.	Hb. %	Erythrocytes Mill.	Leucocytes	Bilirubin mg.%	Heilmeyer iron γ %	Barka iron γ %
May 10	65·000	77	36·8	29	81	4·9	11,000	0·59	30	12
Debility ; very poor general condition. Considerable improvement in course of summer.										
Aug. 17	68·700	68	36·8	12	88	4·7	8,000	0·61	110	Trace

8. M. E., aged 50. *Extensive bilateral, fibrous-cavernous pulmonary tuberculosis.* B.K.++, afebrile. Progressive cachexia, cyanosis, difficulty in breathing and tachycardia (pulse 110 to 120 at rest).

	Sedimentation rate mm.	Hb. %	Erythrocytes Mill.	Leucocytes	−6 TbK −4 mm.	mm.	Bilirubin mg.%	Heilmeyer iron γ %	Barka iron γ %
Mar. 10 ...	52	78	4·6	14,300 Neutro. 65%	5	10	0·74	108	10
Apr. 20 ...	91	68	4·4	8,000 Neutro. 79%	5	15	1·05	72	15
May 8 ...	83	70	4·1	11,000 Neutro. 85%	5	7	0·56	30	Trace

9. F. F., aged 35. *Extensive bilateral fibrous pulmonary tuberculosis.* B.K.++, afebrile; good general condition. Feverish onset 3 years before. For the past 2½ years the disease had been unchanged.

Pulse	Sedimentation rate mm.	–e TbK–i mm.		Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
72	8	3	3	90	5·7	1·18	135	33
Difficulty in breathing after exertion; no cyanosis.								

10. P. H., aged 38. In 1937, *haemoptysis*; left *pneumothorax*, which was maintained to date. Felt well; worked in spite of the pneumothorax. Average as follows:

Hb.	Erythrocytes	Heilmeyer Iron	Barkan Iron
88%	5.0 mill.	140 γ%	30 γ%

11. M. J., aged 63. *Bilateral, fibrous pulmonary tuberculosis, emphysema.* B.K.++, but clinically stabilised. General condition relatively well maintained; breathing difficult after exertion; no cyanosis.

	Sedimentation rate mm.	Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
Breathing difficulty; slight cyanosis						
July 19	23	98	5·4	0·78	120	Traces
Sept. 7	12	103	4·8	—	150	10
Oct. 20	7	89	4·7	0·96	132	10
Well on the road to improvement.						

12. H. A., aged 52. *Bilateral, fibrous-productive pulmonary tuberculosis. Pneumothrax on right* for past year; no breathing difficulty.

	Temp. °C	Sedimentation rate mm.	–e TbK–i mm.	mm.	Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
Mar. 21 ...	38·0	41	4	22	91	4·6	0·71	30	Traces
May 8 ...	37·3	33	2	25	82	4·2	0·63	90	23
July 19 ...	37·0	21	4	30	85	4·1	0·61	50	10
July 28 ...	36·8	27	6	32	88	4·2	0·46	80	14
Condition of debility, felt tired, depressed, without any deterioration of the lung that could be determined clinically.									
Oct. 24 ...	37·1	35	2	20	79	4·6	0·59	22	11

13. L. A., aged 28. *Quiescent cavernous pulmonary tuberculosis.* Sudden dissemination in the right middle zone, with acute progressive attack after haemoptysis.

	Temp. °C	Sedimentation rate mm.	Pulse	Bilirubin mg.%	Heilmeyer iron γ%	Barkan iron γ%
Mar. 22	37·2	9	90	0·88	63	Traces
Treatment: return from sanatorium in a very good general condition.						
Dec. 12	36·5	5	80	0·83	140	66

14. C. P., aged 53. *Bilateral fibrous pulmonary tuberculosis*, which seen by X-ray, occupied about one-third of the pulmonary area. Stationary for past 3 years. Slight pulmonary emphysema. Neither breathing difficulty nor cyanosis at rest.

	Temp. °C	Sedimentation rate mm.	$-^4$ TbK $-^4$ mm.	mm.	Hb. %	Erythrocytes Mill.	Bilirubin mg.%	Heilmeyer iron γ %	Barkan iron γ %
Mar. 22	37·8	13	5	22	98	4·9	1·03	190	35
May 4	36·5	21	3	22	96	4·9	0·56	118	20
June 5	36·5	8	—	—	97	5·4	0·56	145	23
July 27	36·6	6	6	30	96	5·2	0·44	140	12

These few cases, representing the various stages of the different periods of the progressive form of tuberculosis, deserve to be individually discussed.

In *Case* 1 the reduced serum iron content during the humoral reaction to the primary infection is clearly seen as soon as the tuberculin reaction becomes positive, and even before the appearance of a typical X-ray shadow. At the time that the allergic phenomena were most apparent this serum iron content fell still more. As soon as the organism reacted to the infection by a swelling of the glands, and the humoral signs of the process (blood sedimentation and tuberculin reaction) began to recede, the serum iron showed a tendency to return to normal values.

In the acute progressive attack of *Case* 2 the iron sank during the period of infiltration, but it rose considerably as soon as the tissue began to break down. We see in this increase of the serum iron a manifestation of cell destruction with the release of cellular iron and at the same time the sign of a mobilisation of iron for the purpose of evading the serious danger of a development of the destructive process. Actually the tuberculin reaction indicated a tendency to become more definitely positive; there was also an increase of bilirubin.

Cases 3 and 4 show a rapid progressive development with defective body defence (accelerated blood sedimentation, tuberculin reaction comparatively weak). The iron possessed very low values (15 to 20 γ %). In *Case* 4 a general improvement with a violent skin reaction and pronounced leucocytosis was accompanied by a distinct increase of iron.

Quiescent forms of tuberculosis characterised by slow development usually showed a low iron content, which, however, was higher than that of the acute forms. As soon as the X-rays revealed an improvement (*Case* 5) with a reduction of the sedimentation rate and of the skin reaction, we note a conspicuous increase of iron; once the clinical improvement was established this attained normal values.

We note a similar reaction in *Case* 6 in which the slightly

reduced serum iron began definitely to increase from the time of the clinical and humoral improvement.

Cases 7 and 12 were chronic quiescent forms showing a permanently reduced serum iron content.

Cases 9, 10, 11 and 14 correspond to the condition of chronic pulmonary tuberculosis with very extensive lesions involving great reduction of the surface of respiration, which are further complicated by pulmonary emphysema or a pneumothorax. The patients were non-hospitalised cases who often could not avoid being exposed to bodily exertion. Even in a condition of rest these patients suffered from difficulty in breathing, and this was accentuated by the slightest exertion. They regularly presented a normal or even an increased serum iron content. They showed no serious symptoms of inflammation and their difficulty in breathing was due rather to the limitation of the surface of respiration than to the specific pulmonary infection. The normal or increased serum iron content of these cases was the expression of a certain adaptation of the iron metabolism to the laborious conditions of respiration produced by the disease. We shall dwell in detail on this particular problem on pages 197–202.

On the whole, it would be erroneous to consider the concept of a typical serum iron reduction as applying generally to pulmonary tuberculosis. If the individual case of tuberculosis is carefully followed, it can be seen that the value of the serum iron changes in accordance with the stages and the evolution of this disease.

During the period following immediately upon the primary infection, which can be designated as the stage of development of allergy to tuberculin, the serum iron content rapidly declines, but as soon as the organism has organised its defence it gradually increases again. This reduction of iron is probably due to the same cause which *Heilmeyer, Keiderling* and *Stüwe* observed in the case of urticarial rashes provoked by allergy to primroses. Below we shall revert to this problem in connection with certain observations regarding allergy and eosinophilia.

A progressive attack of tuberculosis with clinical symptoms of poor resistance is accompanied by a very great decline of the serum iron content; this same decline may also be observed in most cases of acute inflammatory diseases, especially in those showing a character of infiltration. On the other hand, where there is a break-down of tissue with the formation of cavities, a transient increase of the serum iron content can be noted, whether this be due to tissue destruction or to the reactive mobilisation of the iron enlisted for increased defence.

The chronic forms unaccompanied by acute exacerbations are

characterised, if there is a tendency to improve, by a slightly decreased serum iron content with an occasional short-lived increase of the serum content and stabilisation at normal values. The very chronic forms with a great reduction of the surface of respiration, the cirrhotic forms which are often accompanied by emphysema, and certain cases treated with pneumothorax, all of which are characterised by chronic dyspnoea, frequently show a normal or even an increased serum iron content.

We now come to the discussion of our observations from the point of view of the state of the serum iron under conditions of allergy. In this connection we wish to draw attention to the fact that *Volland* in his experiments on animals injected repeatedly with serum found fairly regularly a distinct reduction of the serum iron content, at the same time as the reticulum was being enriched with iron.

We followed the serum iron curves in a few cases of eosinophilic pulmonary infiltration, as described by *Loeffler*, and on these we were also able to make regular clinical observations.

(1) Miss F. E., aged 19. *Relapsing eosinophilic pulmonary infiltration.* No parasite eggs in stool.

May 17. Small infiltration. Blood sedimentation: 5 mm. Leucocytes 4500, eosinophiles 10%. A-iron=0. B-iron=60 γ%. Rapid disappearance of the pulmonary shadow.

June 9. New infiltration. Reduction of blood cells 5 mm. Leucocytes 6300, eosinophiles 19%. A-iron=0. B-iron=70 γ%. Slow disappearance with progressive decline of eosinophilia.

1 month later: Eosinophilia 7%. A-iron=10 γ%. B-iron=95 γ%.

(2) Miss M. J., aged 24.

June 9. Great infiltration with eosinophilia 11%. Intestinal parasites absent. A-iron=10 γ%. B-iron=70 γ%.

June 16. The infiltration rapidly recedes. Eosinophilia 31%. A-iron=10 γ%. B-iron=70 γ%.

1 month later: Eosinophilia of 4.5%. A-iron=20 γ%. B-iron=120 γ%

(3) Miss C. M., aged 33. *Eosinophilic pulmonary infiltration.* No parasite eggs.

Date	Eosinophilia %	A-iron γ%	B-iron γ%
May 8	16	42	125
May 11	16	47	80
May 15	10	17	88
May 24	Complete disappearance of pulmonary shadow.		
	10	14	117
June 4	11	10	124
June 13	5	16	138

(4) Miss B. E., aged 56 *Eosinophilic pulmonary infiltration.*

May 23	20	38	93
May 29	18	40	103
June 5	14	26	108

In these cases a reduction of the serum iron during the periods of the most severe eosinophilic attacks can be observed, that is,

in the course of a severe allergic manifestation. One is therefore led to assume that this reduction of iron in the acute form of allergy, as in acute infections, must be associated with the activity of the reticulum with which it is connected.

We must not forget what we mentioned previously, that a relatively low percentage of iron in infectious states is not necessarily always the expression of iron mobilisation in the reticulum; it can also be due either to a decrease of the plasma proteins or to a modification of their iso-electric point or to a disturbance of the formation of the globin necessary for the formation of blood pigment.

Iron is thus seen to be an indispensable substance in the defence reaction against infection and is of great service (*a*) for the construction of the white blood cells; (*b*) for catalytic support of the tissue respiration upon which special demands are being made; and (*c*) as an activator of the immunisation processes in the reticulum. However this may be, it would appear that this retention of iron in the reticulo-endothelial system is greatly needed in the fight against infection. If it is absent the prophylactic forces of the organism often break down. The fluctuations of the serum iron in the course of acute allergic phenomena accompanied by pronounced eosinophilia should also be attributed to such a mechanism.

III. Iron Metabolism in Lead Poisoning

The problem of the relationship between iron metabolism and the bone-marrow is particularly important in lead poisoning. Lead poisoning is characterised by two constant phenomena which are of importance for the diagnosis and pathogenesis of this poisoning. These are, first, the presence of a secondary anaemia with basophilic stippling in the erythrocytes, and secondly, marked porphyrin formation. Moreover, porphyrinuria and anaemia are the first two symptoms of the poisoning and they are always present during the condition.

Various authors have observed that there already exists in lead poisoning an exceptionally rich content of porphyrin in the bone-marrow. This porphyrin is a Coproporphyrin III (*Grotepass, Fischer* and *Duesberg-Mertens, Vannotti, Vigliani, Waldenström*), which is closely allied to the haemin of blood pigment. The determination of this fact is of great significance, since in several types of Porphyria the porphyrin formed in the organism belongs to the Isomer group I (*Fischer*) and has no connection with haemoglobin. In lead poisoning, therefore, the porphyrin which has been developed appears to be directly related to the haemoglobin metabolism, i.e. to the function of the bone-marrow.

This hypothesis has been confirmed by a number of investigations which have been undertaken in recent years (*Vannotti*) and we wish here to record these observations in connection with the problem of non-haemoglobin iron. Our experiments have shown that the bone-marrow is the organ which shows the highest percentage of porphyrin concentration in lead poisoning. The fluorescence of this pigment is localised particularly in the erythroblasts, but it can also be seen in the reticulo-endothelial elements, in the bony structure and in the myeloid cells. In the porphyrin metabolism of lead poisoning the liver plays the passive role of an organ of conversion and excretion.

Martini, who has occupied himself particularly with the problem of Saturnism, very correctly associates the presence of the porphyrins with lead anaemia. He is of the opinion that the anaemia is to be attributed to an increase of the porphyrin-forming erythroblasts and to a resulting decrease of the normal red blood cells in circulation. Hence the condition would represent a disturbance of blood regeneration in the sense that the chromogens of the blood pigment are prematurely eliminated by the organism in the form of porphyrin.

This second assumption of *Martini* does not strike us as entirely plausible. Our own observations have shown that the organs of pigment excretion, the kidney and liver, are rather

poor in porphyrins. From this we conclude that the condition is rather one of over-production of porphyrin in the bone-marrow than of a deficiency of the substances needed for haemoglobin synthesis due to premature excretion.

The fact that the porphyrin found in the bone-marrow possesses the same chemical structure as haemoglobin leads to the assumption that there is a disturbance of haemoglobin synthesis, characterised by iron deficiency in the blood pigment molecule. (The porphyrins are chemically differentiated from haemoglobin by the absence of iron in the centre of the molecule.) These conditions lead us to conclude that porphyrin formation in lead poisoning is caused by a disturbance in the metabolism of the haemoglobin iron.

For this reason we undertook a series of experiments with the object of studying the various phases of the non-haemoglobin iron metabolism in the course of lead poisoning. The multiplicity of factors which are able to influence the content of the circulating iron made it difficult to explain the clinical and experimental observations. We endeavoured to overcome this obstacle by following the changes in the iron during certain reactions produced by familiar mechanisms (haemolysis and blocking of the reticulum). Furthermore, we observed the effect of lead on bone-marrow cultures, obtained in the manner described in the preceding section.

The first series of experiments enabled us to follow the iron

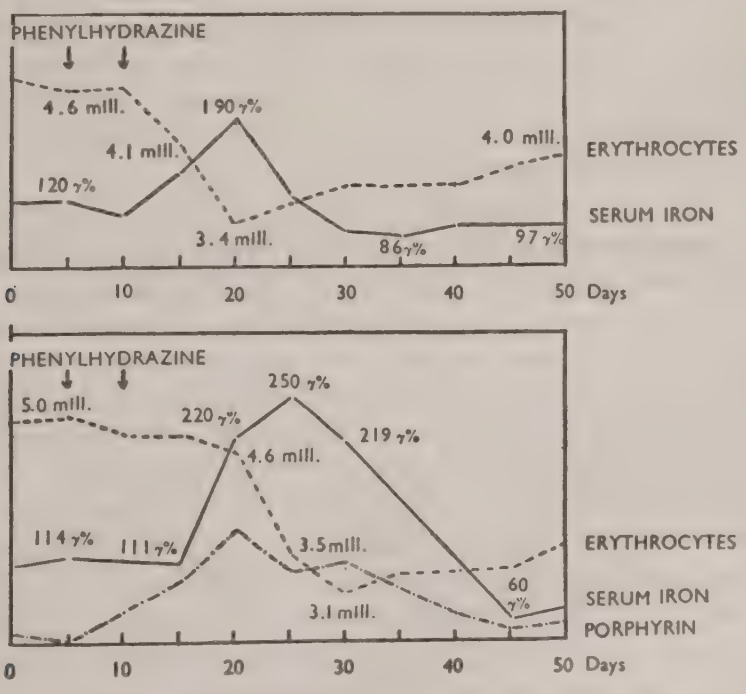

DIAGRAM 7
Phenylhydrazine haemolysis in a case of lead poisoning

curve in the course of haemolysis produced by phenylhydrazine, both in human subjects (occupational poisoning) and in experimental animals which had previously been treated with lead. The greatest attention was given to the post-haemolytic phase, which in normal individuals is characterised by a marked compensatory regeneration of the erythropoietic system. The curves in Diagram 7 illustrate these experiments.

Similar curves were obtained with rabbits (see *Vannotti*: "Porphyrine und Porphyrinkrankheiten"). During haemolysis in the course of lead poisoning the curve of the non-haemoglobin iron rises much higher than that of normal individuals. This curve retains a high level long after that of the control individuals has fallen to below the original values.

A greatly increased serum iron content in the course of haemolysis in lead poisoning is not to be attributed to a possible reduction in resistance on the part of the red blood corpuscles. The degree of anaemia produced, as also the formation of bilirubin and urobilin, remains within the normal limits. The most plausible explanation appears to be the following: The iron liberated by haemolysis in poisoned individuals is not utilised by the bone-marrow, as is normally the case. This situation shows some analogy with that found in aplastic anaemias and in anaemia caused by a blocking of the reticulum, and this would explain the considerable and retarded increase of the serum iron content in the case of these patients, as compared with the slighter and shorter rise in healthy individuals where artificial haemolysis is applied. This tendency to a more protracted increase in the content of the circulating iron during lead poisoning would at the same time explain the existence of great quantities of deposit iron seen to be contained in the tissues in lead poisoning.

We endeavoured to examine more closely the question of the possible utilisation of the iron by the poisoned bone-marrow. With this in view we instituted a number of experiments in the blocking of the reticulo-endothelial system during lead poisoning. We asked ourselves whether this suspension of iron utilisation in haematopoiesis corresponded to a blocking of the bone-marrow reticulum by lead. *Vannotti* and *Imholz* examined the values of the non-haemoglobin iron and of the haemoglobin iron, as well as the number of red blood cells, in rabbits poisoned with lead, whose reticula had been blocked by repeated injections of metals with colloidal pigments. After the rabbits had been poisoned we tried to inhibit the reticulum. In the course of poisoning a secondary anaemia gradually developed, whilst the iron retained its initial value or even showed a tendency to rise. These facts coincided with our former observations, which showed that the

bone-marrow, under the influence of lead, is not able to utilise the iron. Whilst the anaemia was developing a pronounced excretion of porphyrin could be observed in the urine. The injection of substances possessing blocking characteristics produced the following slight reaction on the part of the erythropoietic system: The blood values temporarily increased, and this was followed by a new decline in the number of erythrocytes and in the haemoglobin values, and by a definite exhaustion of the reserves of circulating non-haemoglobin iron. The pathological excretion of porphyrin did not undergo any marked change during the blocking. The exclusion of the reticulum in the course of the lead poisoning did not therefore affect the formation of porphyrin in the bone-marrow; that is, it did not modify the pathological synthesis of this iron-free blood pigment.

The reaction of the bone-marrow is very different if, as in accordance with our procedure in a second series of experiments, the reticulum is blocked before the lead poisoning is initiated. The effect of the blocking is indicated by a slight but progressive anaemia which increases when lead is introduced. The non-haemoglobin iron diminishes, contrary to the condition we found in our previous observations, in which we often noted a tendency on the part of the circulating iron to rise in the course of lead poisoning. Finally, in all the cases in which poisoning had been preceded by a blocking we were able to note the absence of the porphyrin excretion which is so characteristic of lead poisoning. Autopsy disclosed the absence of porphyrins, both in the bone-marrow and in the other organs.

From this we concluded that in lead poisoning the previous blocking of the reticulo-endothelial system prevents porphyrin formation in the bone-marrow and an increase of the circulating non-haemoglobin iron. These determinations permit us to assume that a previous blocking of the reticulo-endothelial system prevents the lead from penetrating to the erythroblasts. The investigations above described regarding the metabolism of iron and blood pigment during the blocking of the bone-marrow reticulum have shown that an obstruction of the reticulo-endothelium never results in an extraordinary amount of porphyrin being produced in the bone-marrow. It must therefore be assumed that the action of the lead on the bone-marrow does not take the form of a blocking of the reticulum. The fact that a blocking occurring after the poisoning does not greatly affect the production of porphyrins, characteristic of Saturnism, whilst previous blocking prevents this pigment from being formed in the bone-marrow, permits us to assume that the point of attack in the bone-marrow for the assault of the lead is the erythroblast itself. This finding is reinforced by

the results of the above-mentioned experiments on iron metabolism in lead poisoning, which show that the iron no longer has a chance of being used by the bone-marrow. The seat of lead poisoning in the bone-marrow is therefore the erythroblast, or it may possibly occur during the transfer of the iron from the reticulo-endothelial cell to the erythroblast.

These observations admirably coincide with the histological observation of a number of erythroblasts which as a result of lead poisoning never attained maturity. Our assumption would furthermore confirm the hypothesis of *Starkenstein* relative to the suspension of the activity of the erythroblast nucleus in the presence of lead. The lead prevents the utilisation of iron for haemoglobin synthesis in the erythroblast; but, on the other hand, the supply of the haemochromogens needed for the formation of the porphyrin nucleus is not affected by lead poisoning, as is the case in the blocking of the reticulum.

Recently, we studied again with *Prader* the problem of lead poisoning with the help of radio-active iron, in relation to the formation of haemoglobin and the cytochrome C content of the tissues. These experiments showed that radioactive iron accumulates during lead poisoning in the bone-marrow, without being utilised. Moreover, a decrease of cytochrome C does not correspond to a decrease of haemoglobin, which shows us that there is no relation between the formation of haemoglobin and cytochrome C. Iron metabolism is then disturbed only at the level of the bone-marrow; it is not modified in relation to the formation of cytochrome C.

A decrease of the haemoglobin corresponds, according to our observations, to an increase of the cytochrome C. This fact is probably due to a compensatory process in the tissues, which shows that iron metabolism is regulated by the functional requirements of the organism.

IV. Iron and the Hyperfunction of the Bone-Marrow

In the preceding chapters we discussed the close co-operation between non-haemoglobin iron metabolism and the function of the bone-marrow. Most of the observations related to processes occurring during the development and in the course of the various forms of anaemia. We shall now proceed to a study of iron metabolism in connection with hyperfunction of the bone-marrow, as, for instance, occurs in polycythaemia (hyperfunction of the erythropoietic system) and in the leukaemias (hyperfunction of the myeloid system).

The secondary polycythaemias will be disregarded at this point; they are dependent upon many diverse factors and are particularly the manifestation, first, of a partially lowered oxygen pressure (of high altitudes, polycythaemia of carbonic acid intoxications), and secondly, of disturbances of circulation and respiration (polycythaemia of cardiac defects, of pulmonary diseases, pneumothorax, etc.). These cases will be studied later in connection with the special conditions which produce this form of polycythaemia. For the present, therefore, we will only consider the polycythaemia vera of *Vaquez* and the leukaemias.

In the single case of polycythaemia vera described by *Heilmeyer* this author noted normal or slightly reduced serum iron values: 87 γ%. In a case of *Geissböck* polycythaemia *Vannotti* observed diminished iron values (1937). These rapidly augmented under the influence of phenylhydrazine treatment, which increased the haemolysis, thereby mobilising a certain quantity of iron.

The following cases confirm on the whole the results of the first authors. But it should be remarked that in nearly all the cases the level of the *Barkan* fraction was comparatively lower than that of the *Heilmeyer* iron. These observations would in part confirm the fact that in polycythaemia there is slight destruction of blood corpuscles (*Eppinger*). But in addition to the reduction of the separable iron we also found a decrease of the total iron and its fractions, which was hardly balanced by the haemolysis which was provoked. Thus in polycythaemia vera we find, besides a certain reduction of the physiological haemolysis, a hyperfunctioning of the bone-marrow. The latter consumes more iron than does normal bone-marrow and thus causes a certain reduction of the serum iron level.

The phenylhydrazine treatment frequently produces a certain increase of iron which, according to *Sachs, Levine* and *Griffith,* is succeeded by an increase of copper. This phenomenon is the expression of increased haemolysis, which is revealed, not only in the serum iron, but also in the iron of the total blood and of the plasma. Nevertheless, it is interesting to note that the phenyl-

hydrazine treatment is not always accompanied by an increased serum iron content. Everything depends upon the degree of the haemolysis produced; weak doses of the drug increase the haemolysis so slowly and so slightly that the rise of the serum iron is not noticeable.

Here are a few examples:

G. E., Jan. 20, 1942.

Hb. %	Erythrocytes Mill.	Leucocytes	Sedimentation rate mm.	A iron γ%	B iron γ%	C iron γ%	D iron γ%	Total iron γ%
112	9.780	30,000	0	0	115	15	very pronounced haemolysis	1125

B. E., aged 69.

	Hb. %	Erythrocytes Mill.	Leucocytes	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Dec. 3, 39	142	8.5	27,600	Traces	56	0.13
Dec. 10, 39	Phenylhydrazine, 0.1 Nr. VI.					
Dec. 10, 39	112	7.2	17,000	10	84	0.2
	No treatment.					
Mar. 2, 40	114	8.4	26,800	15	125	0.13
	Venesections, diet.					
Nov. 28, 40	109	7.8	29,000	Traces	96	0.11
	Phenylhydrazine.					
Dec. 2, 40	105	6.2	27,000	47	279	0.11

B. L., aged 50.

	Hb. %	Erythrocytes Mill.	A iron γ%	B iron γ%	C iron γ%	D iron γ%	Total iron γ%
Feb. 6, 42	115	9.7	0	110	95	50	255

B. F., aged 45.

	Hb. %	Erythrocytes Mill.	Reticulocytes %	Bilirubin mg.%	A iron γ%	B iron γ%	C iron γ%	D iron γ%	Total iron γ%
May 23	98	6.9	4	0.35	25	123	82	40	270
	Rp. Phenylhydrazine 0.1 g. on 1st day, then 0.2 g. for 9 days								
May 28	97	6.180	7	0.25	60	130	40	60	290
June 2	87	5.3	9	0.51	76	119	105	74	374
	End of treatment.								
June 4	87	5.530	8	0.35	70	85	50	22	227

A very energetic phenylhydrazine treatment (1.9 g. in ten days) produced only a relatively feeble haemolysis. Probably the patient possessed a certain resistance towards the drug, as a result of previous treatment with phenylhydrazine. The blood regeneration was active. As early as two days after the end of treatment the number of red blood cells already showed a tendency to rise and the total serum iron was lower than at the beginning. Here the bone-marrow seemed to be chiefly utilising the fractions of the easily split-off iron. The bilirubinaemia appeared hardly to rise at all, which permitted the assumption that the haemoglobin decomposition did not proceed in the usual way.

T. R., aged 48. *Mild case of Cushing's Disease.*

	Hb. %	Erythro-cytes Mill.	Leuco-cytes	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Oct. 27	113	6.7	7400	Traces	60	0.4
	Phenylhydrazine.					
Nov. 2	100	6.4	7500	10	95	0.4
Nov. 8	93	4.9	5870	41	178	1.2
Nov. 29	95	5.1	6900	Traces	52	0.3
Dec. 6	115	6.7	55900	Traces	60	0.3
Feb. 17	108	6	8200	Traces	65	—
	X-ray irradiation of pituitary body.					
Mar. 14	100	5.3	5400	12	82	0.5
Apr. 11	85	5.2	7000	12	97	0.7
May 25	85	5.3	7000	15	90	0.91
Aug. 4	85	4.7		10	41	0.93
	End of X-ray treatment.					
Aug. 28	91	5.2	5700	10	80	1.22
Sep. 19	90	5.7	5400	15	90	0.90
	R.X. one sitting.					
Sep. 22	90	5.140	4300	21	105	0.88
Sep. 25	90	5.110	4300	1	86	1.08
Nov. 5	86	4.640	3500	1	61	0.51

In the last case disturbances of the hormonal regulatory mechanism involved, among other clinical manifestations, hyperactivity of the bone-marrow. As a result of increased iron consumption the serum iron content was low; on the other hand, the blood destruction was not very active, as is shown by slightly increased bilirubinaemia. Under the influence of the phenylhydrazine we observed increased haemolysis, which was characterised by a rise of the serum iron (the *Barkan* iron increases from traces to 41 γ%, the *Heilmeyer* iron from 60 γ% to 178 γ%) and of bilirubinaemia; but immediately after the end of the treatment these values rapidly descended to normal again, even passing through a first phase with greatly reduced serum iron (*Heilmeyer* iron = 52 γ%), which is probably to be attributed to reactive blood

formation. The effect of X-rays on the pituitary region was but slight as regards the serum iron, which remained at a little below normal values. The small decrease of the Hb. level and of the number of erythrocytes was in this case not connected with greater blood destruction (non-haemoglobin iron level unchanged).

A factor of great importance in leukaemia has been the examination of the non-haemoglobin iron, ever since *Amano* and his followers introduced a system of sub-dividing the blood cells according to their content of certain iron-containing pigments. On the basis of detailed spectroscopic observations *Amano* reached the conclusion that the blood cells of vertebrates, considered from a phylogenetic and ontogenetic point of view, belong either to a "haemoglobin type" (the red blood cells) or to a "cytochrome type" (the white blood corpuscles). Moreover, this author has shown that the irreversible hyperplasia of one of these haemopoietic systems involves the reduction, and even the disappearance, of the pigment characterising that particular cellular system. Thus, aplastic anaemia would be the manifestation of a disturbance in haemoglobin formation, and agranulocytosis that of a severe alteration in cytochrome formation.

In human pathology, according to *Amano,* the appearance of young and malignant blood cells in the bone-marrow and the circulation, as observed in erythroblastosis or in the leukaemias, is characterised by a loss of the pigment typical of each of the two cellular systems (haemoglobin for erythroblastosis, cytochrome for leukaemia). This concept would explain the absence of the Nadi reaction in the myeloid cells in leukaemia.

In the course of leukaemia, therefore, the serum iron content is a matter of great interest. *Heilmeyer* and *Plötner* have shown that the iron value may vary in leukaemia. Sometimes it is reduced, but in most cases it is normal or increased. The rise is particularly obvious at the time of the destruction of the white blood cells by X-rays; this fact led these authors to assume that the leucocytes contained iron. Nevertheless, leukaemia is often accompanied by severe secondary anaemia, which may result in a reduction of the serum iron. On the other hand, the influence of the X-rays on the activity of the bone-marrow assumes two forms, viz. inhibition of haematopoiesis and stimulation of blood destruction. The increase of serum iron in cases of leukaemia treated by X-rays may be partly the manifestation of an induced haemolysis. The continuation of a somewhat higher iron value permits us to support the view of the two above-mentioned authors that the phenomenon is to be attributed to very active cellular metabolism, and particularly to increased destruction of the white cells. These observations thus enable us to admit the possibility that the white

blood cells may contain non-haemoglobin iron. This iron is bound to the nuclear substance, as in the cells of the other tissues. Part of it belongs to the cytochrome which is conveyed to the circulation by the leucocytes. According to this theory this pigment suffers the same fate as the haemoglobin; it is destroyed in the liver and is even built up from the same synthetic substances as the blood pigment (*Amano*). Increased destruction of the white blood cells brings about a similar rise of serum iron as in the case of the red blood corpuscles, since haemoglobin and cytochrome, which possess a similar constitution, probably present an analogous cycle of synthesis and destruction.

Here are a few observations of cases of leukaemia:

M. M., aged 64. *Chronic myeloid leukaemia*, very pronounced. Splenomegaly, pains in the bones, especially in the wrists and ribs. General condition comparatively good.

A few weeks after X-ray treatment:

	Hb. %	Erythrocytes Mill.	Leucocytes	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
Jan. 11	60	4.1	12,100	Traces	32	0.54
Jan. 15	60	4.2	29,600	Traces	26	0.66
Feb. 7	60	4	51,600	Traces	10	0.61

The general condition of the patient continued to deteriorate; the serum iron content which had at first diminished, in accordance with the exacerbation of the leukaemia, gradually increased in the course of the next two months, during which time a leucocytosis of 140,000 developed.

	Hb. %	Erythrocytes Mill.	Leucocytes	Barkan iron	Heilmeyer iron γ%	Bilirubin mg.%
Dec. 27	54	3.05	137,000	Traces	82	0.83
	Six months later fairly rapid deterioration.					
June 21	52	2.99	220,000	Traces	36	

Second combined treatment with X-rays and arsenic, resulting in considerable improvement. After six months the patient returned for observation. The general condition was moderate. Blood examination showed the following values:

	Hb. %	Erythrocytes Mill.	Leucocytes	Barkan iron	Heilmeyer iron γ%	Bilirubin mg.%
Dec. 16	52	3.40	70,000	Traces	80	0.15

The determination according to our fractional technique was:

A-iron ..	0 γ%
B-iron ..	100 γ%
C-iron ..	20 γ%
D-iron ..	135 γ%
Total iron ..	255 γ%

The partition is the same as seen in essential hypochromic anaemia.

B. A., aged 16. *Acute myeloid leukaemia.* Severe general condition. Died 3 weeks after the following blood examination:

Hb.	Erythrocytes	Leucocytes	Bilirubinaemia		
52%	2.50 Mill.	560,000	0.15 mg.%	A-iron ..	0 γ%
				B-iron ..	200 γ%
				C-iron ..	10 γ%
				D-iron ..	30 γ%
				Total iron ..	240 γ%

B. B., aged 66. *Chronic lymphatic leukaemia. Splenomegaly.* Abundant swelling of lymphatic glands. No fever.

Hb. %	Erythro-cytes	Leuco-cytes.	Barkan iron γ%	Heilmeyer iron γ%	Bilirubin mg.%
63	3.00 Mill.	14,000 of which 92% were lymphocytes	10	109	0.51

C. C., aged 70. *Chronic lymphatic leukaemia. Splenomegaly, diffuse glandular swelling.* Good general condition, no fever.

	Hb. %	Erythro-cytes Mill.	Leuco-cytes	Barkan iron	Heilmeyer iron γ%	Bilirubin mg.%
Jan. 6	50	3.07	44,200	Traces	41	0.54
Jan. 22	60	3.85	33,000	Traces	115	0.56

Thus we can confirm the data contained in the literature indicating that the iron value usually reflects the course of the leukaemia.

The leukaemias characterised by exceptionally slow evolution often have a normal iron content or one corresponding to the degree of the attendant anaemia, and for this reason a slightly reduced iron content. Moreover, we think it of interest to mention a case of myeloblastic leukaemia in which *Büchmann* found

distinct reduction of the iron, especially during the febrile period (to the extent of complete disappearance of the serum iron). The author considers this decrease to be a secondary phenomenon, due to the fever. The reduced serum iron content that follows the infection is, as we have seen, a well-known phenomenon and corresponds to a mobilisation of iron for the purpose of activating cell metabolism and of increasing the prophylactic measures of the body. This explanation is intelligible, but it might still be asked whether in cases where the destruction of a great number of myeloblasts would not tend to augment the serum iron content such an exceptional reduction of iron might not rather be indicative of a failure on the part of the iron to fulfil its general biological functions associated with general metabolism. This would mean that the catalytic iron would be consumed by the periphery, whilst the greatly changed white cells, having been deprived of cytochrome, would cease to have any biological significance.

Recently, *Cartwright, Wintrobe* and their collaborators described seven cases of leukaemia and Hodgkin's disease with a reduced iron percentage and eleven cases with a normal percentage of non-haemoglobin iron.

In leukaemia the considerable formation of pathological immature blood cells is not accompanied by a corresponding enrichment of cytochrome; from now on the iron only takes part in leukopoiesis as a nuclear constituent of the white blood cells. Thus the increase of the serum iron would result both from impaired utilisation of this metal and from greater destruction of the myeloid cells. In myeloid leukaemia we are confronted by a dangerous anomaly of iron metabolism, for the bone-marrow is no longer able to form leucocytes provided with the iron-containing pigment needed to carry out the important functions of these cells. The conditions are those found in tumours where, according to *Warburg* and his followers, there is a great diminution of biological catalysts based on iron and where oxidation makes way for glycolysis. In this comparison we see a certain analogy with the opinion of those authors who interpret leukaemia as a special form of tumour.

We also wish here to mention the observations of *Stodtmeister* and *Büchmann* regarding five cases of leukaemia. They found that when the leukaemia became worse the serum iron content declined, irrespective of the haemoglobin value and the number of erythrocytes; also that destruction of the white blood cells after treatment with X-rays was not always followed by an increase of iron. This observation contradicts the findings of *Heilmeyer*, who ascribes the increased serum iron content to the destruction of the white blood cells. If a series of X-ray treatments results in clinical improve-

ment there can usually be observed an increase of the serum iron value; on the other hand, a lower iron content after X-ray treatment is always the sign of a bad clinical reaction to the treatment.

Skouge described a case of acute myeloid leukaemia in a girl of 12, who showed an increased serum iron content. *Vahlquist* also observed a relatively high iron value in two cases of leukaemia in children.

Stodtmeister and *Büchmann* see a certain analogy between the anaemia which usually accompanies leukaemia and that form which appears in the course of infectious conditions where iron is rapidly mobilised for the prophylaxis of the organism. Actually, the anaemia of leukaemia is preceded by a long period during which the serum iron is greatly reduced. This would therefore primarily be a condition of iron-deficiency anaemia. This first phase would later on be followed by anaemia caused by the myeloid infiltration of erythropoietic tissue.

The failure of iron metabolism is, therefore, in the opinion of these authors, a characteristic symptom of the exacerbation of the leukaemia. This assumption would agree in principle with what we have just said regarding myeloid leukaemia. We must repeat that we are here groping in the domain of hypothesis, but these few remarks may be of some value in clarifying the important role played by iron in connection with the myeloid function. At all events, it can be assumed that a certain rise of the serum iron value in leukaemia is a phenomenon frequently observed, and that a great and rapid reduction of this value in the course of the disease is a sign of deterioration.

We must add here the experiments of *Wintrobe* and his collaborators on the polycythemia provoked by cobalt. This metal favourably influences the utilisation of iron for the synthesis of haemoglobin.

Lastly, it is useful to recall here that another metal, copper, plays an important but not yet understood part in the formation of the haemin. *Elvehjiem*, his collaborators and *Schultze* showed that copper is necessary for the mobilisation of iron from the tissues and for its conversion into haemoglobin. The same authors observed also that copper is essential for the formation and the activity of cytochrome, cytochrome oxidase and catalase. It is possible that copper acts as a catalyst for the introduction of iron into the porphyrin ring of the " haems ", or that copper acts indirectly on the synthesis of haemoglobin by stimulation of the cytochrome oxidase activity of the bone-marrow.

In our institute, *Suriyong*, in the treatment of polycythemia with nitrogen mustard could show that the serum iron increases

considerably after the treatment which provoked an inhibition of the marrow. This fact shows that the increase of the spleen activity continues for some weeks after the interruption of the erythropoietic hyperactivity of the marrow.

V. Iron in Hepatic Diseases

The liver plays an essential role in iron metabolism. In its reticulo-endothelial system it decomposes haemoglobin into bile pigment by breaking the porphyrin ring of the blood pigment and liberating the iron. According to *Starkenstein* and *Weden* the reduction of the ferric salts to ferrous salts is effected in the liver with the help of the reticulo-endothelial system. But this reduced iron cannot serve as a biological catalyst (it does not possess the properties of an "active" iron) and it is more suitable for the formation of blood pigment. The liver can also be considered as an organ of storage for this released iron.

Primarily, therefore, the liver carries out two quite distinct functions in iron metabolism: first, it takes part in the mechanism of haemolysis, and secondly, it takes up the oxidised iron which has just been used in the tissues as a biological catalyst and prepares it for use in haemoglobin formation. We wish to take this opportunity of drawing attention to the conclusions reached by *Hahn*, who views the copper in the blood and tissues as a catalyst which makes it possible for the iron deposited in the liver to be utilised. Thus the hepatic parenchyma is destined to play a considerable part in physiological haemolysis and a still greater part in all the pathological processes of blood decomposition. It is the activity of the liver that determines the amount of iron liberated during haemolysis.

We have already mentioned the relationship existing between iron and haemolysis; here we wish again to emphasise the fact that a functional disturbance of the liver, affecting the hepatic reticulum, may have a certain effect on iron metabolism.

As stated, the supply to the bone-marrow of the iron needed for blood pigment formation depends, in part at least, upon the hepatic parenchyma. A certain proportion of the divalent iron needed for haemoglobin formation in the bone-marrow is actually prepared in the liver. Finally, it must not be forgotten that, according to *Starkenstein* and *Weden*, the reduced inactive iron derived from the various converted forms of inorganic iron, which is to be regarded as the final product in the chain of iron metabolism, is deposited in the liver prior to excretion. According to *Starkenstein* and *Weden* the hepatic iron in its oxidised form is insoluble in water and can only be extracted after boiling with 5N HCl. The hepatic cell possesses the power of reducing the iron by enzyme action. The ferrous salts cannot be oxidised in the presence of hepatic tissue, although in the case of blood the opposite conditions apply. Hence the trivalent iron of the liver must be oxidised outside this organ, and it thus takes the form of iron fixed in the process of

passing through the liver. In this way the liver is made to play diverse roles in iron metabolism, thanks to the function of the hepatic cell and the reticulum, which makes it both a centre for iron conversion and an organ of iron storage and excretion.

The investigations in vitro designed to study the effect of the hepatic parenchyma on haemoglobin showed *Vannotti* and *Siegrist* that parallel with the destruction of the blood pigment there is definite bilirubin formation by the liver, accompanied simultaneously by a great rise of the iron content in the cultures. The co-operation of this organ in the liberation of the haemoglobin iron, independent of the spleen and of other organs participating in haematopoiesis, is thereby proved. The observation made in human pathology of the great iron content in the liver during blood decomposition made the hypothesis appear probable that the liver was directly active in separating the haemoglobin iron and in depositing it in that organ. Finally, we wish to draw attention to what was said in a former section regarding the role of the reticulo-endothelial system and particularly of the hepatic reticulum in connection with iron metabolism, namely, that the iron content is associated with the stimulation or blockage of the Kupffer cells.

The excretion of iron by the liver presents a particularly interesting problem. A certain amount of the iron passes from the liver into the bile. Most authors assume that the amount of iron eliminated through the bile is 1 mg. in twenty-four hours; *Eppinger*, however, found a value of 10 mg.

We have again taken up this question and were able to determine that iron excretion through the bile is very regular. In normal individuals the iron concentration here does not greatly vary within the space of eight hours, and remains at a uniform value of about 0.1 mg.%. As the bile in the gall bladder is more concentrated it rises higher, viz. to an average level of 0.13 to 0.20 mg.%. Under the influence of powerful iron administration (10 mg.) bile elimination does not show any change during the next six hours. Thus the iron excretion through the bile does not appear to be correlated either with the blood iron content or with the immediate introduction of this metal into the organism.

A second point of importance resulting from our analyses is that the iron excreted through the bile is chiefly in an easily split-off form. On the other hand, the bile contains only small quantities of non-separable iron or iron compounds which one might be induced to consider, in part at least, as waste products of iron metabolism. The value of the iron varies according to the fractions of the bile (duodenal bile, gall-bladder bile, hepatic bile). Furthermore, we must stress the distinct difference in the content of bile iron as found in various species of animals.

We give the technique employed for bile extraction:

The stomach of a fasting person is washed out with distilled water in order to remove the remains of food, the presence of which might falsify the iron analyses. The stomach tube is withdrawn and a duodenal tube furnished with a copper ampulla is introduced into the duodenum, after which a slender stomach tube is also introduced. The extremities of the two tubes must each be at a distance of 15–20 cm. from the pylorus, upwards and downwards, respectively. A continuous drainage through the stomach tube makes it possible to remove all the gastric secretions and to prevent any bile from mingling with the gastric juice. For our iron determinations we do not use the "duodenal bile", a bile mixture consisting of gastric juice and intestinal juices, since its composition depends upon too many factors and conditions to enable comparable iron values to be obtained. Instead, we analyse the iron values of the gall-bladder bile, obtained by the injection of 1 cc. of Pituitrin, and also the liver bile.

The following are the results obtained in the case of healthy individuals:

	Gall-bladder bile		Hepatic bile	
	γ%	γ%	γ%	γ%
A-iron ..	(*a*) 21	(*b*) 18	(*a*) 12	(*b*) 10
B-iron ..	142	128	54	66
C-iron ..	70	24	71	19
D-iron ..	11	30	5	5
Total iron ..	254	200	142	100

The iron content of the gastric juice is about the same as that of the hepatic bile:

A-iron ..	(*a*) 39 γ%	(*b*) 25 γ%
B-iron ..	88 γ%	72 γ%
Total iron	110 γ%	92 γ%

For the purpose of comparison we now give the results of the analyses of the iron content in the bile of different types of animals:

	Dog	Calf		Pig
	γ%	γ%	γ%	γ%
A-iron ..	120	10	5	15
B-iron ..	115	78	55	100
C-iron ..	100	87	140	100
D-iron ..	20	7	5	20
Total iron	355	182	205	235

The bile of carnivorous animals contains more iron than that of herbivorous animals. This increase is doubtless due to a greater intake of iron in the food, since the serum iron level in these two groups of animals is not very different. We are therefore inclined to assume that the quantitative difference between the iron values of the bile and the total iron absorbed through the digestive tract is closely related. According to this view the iron elimination through the bile would chiefly affect certain fractions of the nutrient iron.

We were curious to know whether possibly other mechanisms of iron metabolism in the organism might also influence iron excretion in the bile. We followed the bile iron excretion during increased haemolysis and erythropoiesis stimulation in a case of pernicious anaemia. The results of the iron analysis before and during haemolysis induced by phenylhydrazine will be given later.

H. C., aged 35. *Pernicious anaemia.*

	May 10 (before treatment) Hb. 48% Erythrocytes 1.61 mill. Bilirubin 2.5 mg.%		June 9 (after treatment) Hb. 72% Erythrocytes 3.90 mill. Bilirubin 0.05 mg.%	
	Gall-bladder bile	Liver bile	Gall-bladder bile	Liver bile
A-iron ..	17 γ%	72 γ%	10 γ%	10 γ%
B-iron ..	21 „	26 „	60 „	55 „
C-iron ..	92 „	42 „	60 „	50 „
D-iron ..	10 „	5 „	12 „	5 „
Total iron	170 „	145 „	145 „	120 „

We found that increased haemolysis might involve increased iron excretion in the bile. We therefore came to the conclusion that the excretion of iron through the liver appeared to be regular and fairly constant for each type of animal; but that it might change quantitatively and possibly also qualitatively as a result of the introduction of iron through the food, especially under the influence of haemolysis. This observation is supported by the experiments of *Hahn* and his collaborators, who in their investigations on the metabolism of radio-active iron found an increase of this iron in the bile during haemolysis.

The question now arose whether any other physiological or pathological factors might influence the excretion of iron in the bile. In this connection we desire to draw attention to the works of *Hemmeler* concerning the problem of serum iron in the hepatic diseases. Proceeding from the fact that bile secretion

represents one avenue for iron elimination from the organism, this author asked himself whether disturbances of the bile secretion might not also be accompanied by a disturbance of the iron excretion in the bile, that is, by a certain amount of retention of this metal in the organism.

In icterus *Hemmeler* determined the serum iron value according to the *Heilmeyer* method and found that with one exception the serum iron value was not increased in his cases of mechanical icterus (gall-stones and carcinoma). On the other hand, he found that in catarrhal icterus (and we can add, also in epidemic icterus) there occurred with admirable regularity a distinct rise of the serum iron value (an increase which had previously been determined by *Warburg* and *Krebs* and by *Locke, Main* and *Rosbach,* and later confirmed by *Vahlquist, Skouge* and *Waldenström,* although not with the uniformity described by *Hemmeler*). The last author comes to the conclusion that in catarrhal icterus the disturbance of the bile elimination is accompanied by iron retention; but in the case of iron this is only manifested very slowly and not until the bilirubin retention has already been noticeable for several days. The iron starts increasing in the serum at the time that the bilirubinaemia begins to decline. This close relationship between the curves of the bilirubin and of the iron in the serum is not confirmed in the observations of *Vahlquist* and *Skouge*. Intravenous injection of iron to patients with icterus simplex gives the same serum iron curve as in healthy individuals.

Hemmeler explains his results as follows: In all cases of obstruction to bile excretion there is iron retention in the organism; this is mainly visible in catarrhal icterus (which is the consequence of hepatitis). In cases of mechanical bile retention the increase of serum iron is not noticeable, as the basic cause of this retention (neoplasms or gall-stones) in any case provokes a distinct reduction of the serum iron value. As is well known, tumours and infectious or chronic-inflammatory conditions have a general tendency to reduce the serum iron content.

In mechanical icterus the iron retention would compensate for the reduction of the serum iron value. This, according to *Hemmeler,* would explain the presence of a normal iron value in the serum in mechanical icterus. In icterus catarrhalis, on the other hand, in which an injury of the hepatic cell impedes the excretion of the bilirubin and iron before their passage into the bile-ducts and in which these substances accordingly enter the circulation directly, there results in the organism iron retention with a corresponding increase of the serum iron content.

This explanation does not strike us as altogether convincing, for the following reasons: If we assume that the presence of an in-

fection of the bile-ducts or a tumour in mechanical icterus balances the increased serum iron content caused by bile retention, the same phenomenon would be noted to a still greater extent in simple icterus and in the various forms of hepatitis; for the latter conditions present the clinical and anatomico-pathological picture of an inflammation both of the liver and of the total organism much more frequently than do the two above-named forms of disease.

In icterus simplex the iron increase in the serum occurs later than that in the bile, sometimes even only after the bilirubinaemia has again fallen to normal values. The successive retention of these two substances would not explain the transfer of the bile and iron into the blood in the course of icterus caused by hepatitis. In order to explain this different behaviour *Hemmeler* assumes that the iron excretion through the bile-ducts persists after the bilirubin has already been retained in the organism. We should therefore have to assume that at the start of the icterus there was a secretion of bile without bilirubin but with iron, and that towards the end of the icterus there was bile with bilirubin but without iron.

This dissociation in the face of retention of both substances in the course of the hepatitis does not strike us as very probable, all the more so since, according to *Eppinger,* the relationship of the concentration of the bilirubin and iron in the bile during catarrhal icterus shows a slight reduction of the bilirubin excretion and a normal excretion of the iron. This last circumstance would therefore definitely contradict the hypothesis of iron retention in simple icterus.

We resumed these investigations systematically, with the idea of solving this problem by using our fractional analyses of iron. Our attention had previously been directed to an increase of the non-haemoglobin serum iron in icterus catarrhalis.

Here to begin with are two cases of catarrhal icterus, in which we followed the iron metabolism, using the methods of *Barkan* and *Heilmeyer*:

D. M., aged 21.

	Bilirubin mg.%	Barkan iron γ%	Heilmeyer iron γ%
At beginning of illness ..	1.99	64	120
6 days later	2.70	100	135
A. G., aged 23.			
At beginning of illness ..	2.82	144	228
6 days later	1.94	140	143
9 days later		200	168
15 days later	0.96	170	126
1 month later	0.52	97	112

Here are other cases of icterus in which we examined the iron metabolism with our fractional methods of determination:

M. R., aged 20. *Catarrhal icterus* (see Diagram 8).

		Iron γ%	Iron in bile γ%	Serum-bilirubin mg.%
4th day of illness	A-iron	235		2.7
	B-iron	115		
	C-iron	30		
	D-iron	30		
	Total iron	410	168	
11th day of illness	A-iron	142		7.6
	B-iron	218		
	C-iron	110		
	D-iron	170		
	Total iron	640	140	
18th day of illness	A-iron	105		0.9
	B-iron	345	55	
	C-iron	0	100	
	D-iron	0		
	Total iron	450	155	
25th day of illness	A-iron	14	0	
	B-iron	221	20	
	C-iron	100	200	
	D-iron	115	0	
	Total iron	450	220	
32nd day of illness	A-iron	55		
	B-iron	190		
	C-iron	75		
	D-iron	50		
	Total iron	370		

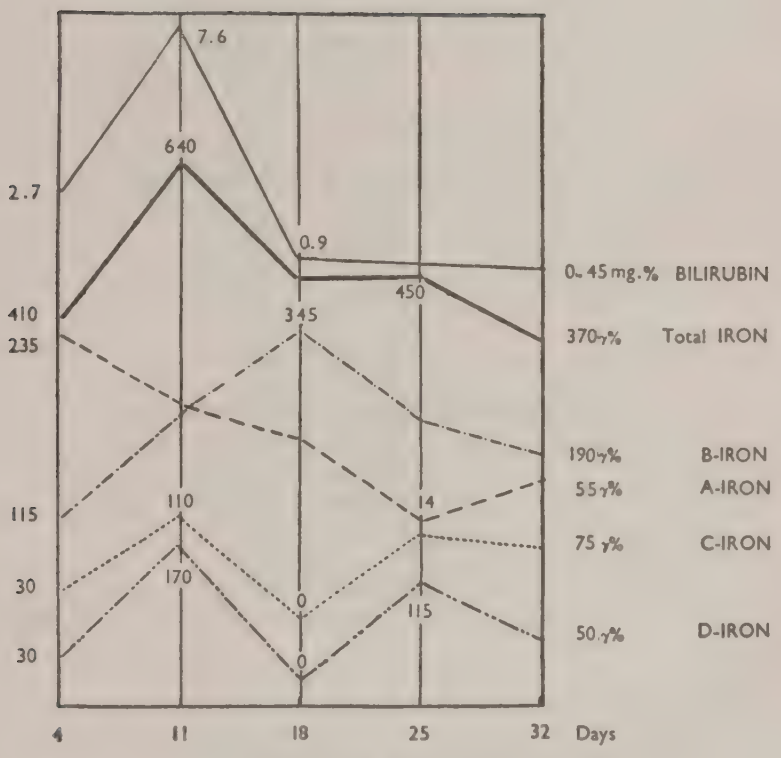

DIAGRAM 8
Serum iron and serum bilirubin curves in a case of catarrhal icterus.

C. B., aged 47. *Chronic catarrhal icterus.*

Serum-bilirubin:	4.4 mg.%
A-iron	33 γ%
B-iron	172 γ%
C-iron	100 γ%
D-iron	100 γ%
Total iron	450 γ%

6 days later. Serum-bilirubin: 4.3 mg.%

A-iron	85 γ%
B-iron	150 γ%
C-iron	55 γ%
D-iron	70 γ%
Total iron	360 γ%

13 days later, after 4 days' menstruation

A-iron	15 γ%
B-iron	155 γ%
C-iron	50 γ%
D-iron	56 γ%
Total iron	276 γ%

12 days later. Serum-bilirubin: 1.0 mg.%

A-iron	60 γ%
B-iron	130 γ%
C-iron	90 γ%
D-iron	115 γ%
Total iron	405 γ%

Miss M. J., aged 45. *Hepatitis acuta with severe icterus.*

8th day of illness	A-iron	60 γ%
	B-iron	240 γ%
	C-iron	50 γ%
	D-iron	50 γ%
	Total iron	400 γ%

T. A., aged 43. *Catarrhal icterus.*

Serum-bilirubin:	3.3 mg.%
A-iron	140 γ%
B-iron	180 γ%
C-iron	110 γ%
D-iron	95 γ%
Total iron	525 γ%

B. A., aged 32. *Catarrhal icterus.*

A-iron	40 γ%
B-iron	225 γ%
C-iron	75 γ%
D-iron	50 γ%
Total iron	390 γ%

S. P. R., aged 43. *Catarrhal icterus.*

Serum-bilirubin: 4.0 mg.%.		1 week later: improved.
A-iron	0 γ%	0 γ%
B-iron	160 γ%	160 γ%
C-iron	230 γ%	75 γ%
D-iron	10 γ%	95 γ%
Total iron	400 γ%	330 γ%

M. F., aged 61. *Catarrhal icterus*, which developed at the end of a chronic inflammation of the liver.

6th day of icterus. Serum-bilirubin: 4.4 mg.%

A-iron	55 γ%
B-iron	190 γ%
C-iron	50 γ%
D-iron	85 γ%
Total iron	380 γ%

10 days later. Serum-bilirubin: 1.25 mg.%

A-iron	20 γ%
B-iron	220 γ%
C-iron	40 γ%
D-iron	90 γ%
Total iron	370 γ%

10 days later. Serum-bilirubin: 0.7 mg.%

A-iron	10 γ%
B-iron	220 γ%
C-iron	90 γ%
D-iron	40 γ%
Total iron	360 γ%

We now give the results of the fractionally-determined iron analyses in a case of mechanical icterus, produced by obstruction of the bile-duct by a tumour of the head of the pancreas.

G. F., aged 47. *Mild secondary anaemia*, Hb. 60%.

Serum-bilirubin: 3.05 mg.%

A-iron	10 γ%
B-iron	60 γ%
C-iron	115 γ%
D-iron	70 γ%
Total iron	255 γ%

A. A., aged 68. *Icterus* after obstruction of bile-duct caused by a carcinoma. Increasing cachexia. Icterus for the past 2 months.

Blood picture: Haemoglobin, 77%; Erythrocytes, 4,600,000; Leucocytes, 6,100; Bilirubinaemia, 9.8 mg.%.

A-iron	0 γ%
B-iron	100 γ%
C-iron	110 γ%
D-iron	30 γ%
Total iron	240 γ%

In a third case of mechanical icterus due to obstruction of the bile-duct produced by a tumour of the head of the pancreas accompanied by severe hepatic insufficiency, the determination of the four fractions of the serum iron gave the following results:

A-iron	0 γ%
B-iron	270 γ%
C-iron	30 γ%
D-iron	30 γ%
Total iron	330 γ%

In this special case, in which we found the simultaneous existence of mechanical icterus and injury to the hepatic cell, we found that the total iron was normal with a relative increase of the closely bound iron. This was therefore a case not of absolute iron retention, but of inhibition in conversion of its fractions.

These observations constitute a new contribution to the study of hepatic-iron metabolism, for they offer us the following data regarding the fluctuations of the various fractions in cases of icterus of varying aetiology.

Above all we confirm the observations of *Hemmeler* relative to the definite increase of the non-haemoglobin serum iron content in most cases of mechanical icterus.

But as a result of our own investigations we can supplement this information by asserting that this increase of the serum iron does not occur only in catarrhal icterus, but also in hepatitis epidemica (infectious icterus). In simple icterus the increase in iron is related not only to the *Heilmeyer* fraction, but is revealed also in the total serum iron and in the other fractions (especially in A and B iron).

Hence we must assume that there is a definite increase of the total iron in circulation in icterus simplex. As the patient's condition improves this increase gradually diminishes, and at the end of the icterus or a few days later the values are normal again.

We will now consider the distribution of this increased serum iron among the various fractions. The following table shows the

total serum iron in absolute values and its fractions in percentages in normal individuals and in a few cases of catarrhal icterus.

	Average distribution of iron of various fractions in normal persons (average of women and men)	Percentage iron distribution of various fractions			
		In some cases of catarrhal icterus			In one case of mechanical icterus
	γ%	γ%	γ%	γ%	γ%
A-iron	5	57	27	22	4
B-iron	50	27	34	40	24
C-iron	33	8	21	19	45
D-iron	12	8	18	19	27
Total iron	283	410	525	360	255

In studying this table we are struck by the following fact: Very often catarrhal icterus shows, in addition to an abnormally increased value for the total iron, a distinct increase in the two fractions of the separable iron, sometimes at the expense of the non-separable iron and of the iron of the protein precipitate. Actually these two fractions (C and D) are either normal or reduced in comparison with the increase of the A and B fractions. Mechanical icterus, on the other hand, tends to be characterised by a decrease of the A and B fractions and by a relative increase of the C and D fractions.

In catarrhal icterus the disturbance of the iron metabolism can be explained in the three following ways: (*a*) Increased intake of the separable iron, (*b*) arrest in the conversion of the separable into non-separable iron, and (*c*) diminished opportunities for utilising or depositing the iron.

In discussing iron metabolism during haematopoiesis we described the occurrence of an increase of the iron fractions A and B in haemolysis and pernicious anaemia. But in haemolysis, especially in the acute form, there was a corresponding iron increase in the protein precipitate, whilst pernicious anaemia showed a corresponding reduction of the C and D fractions, so that the total iron was generally found not to be increased. In hepatitis, on the other hand, we often see a marked increase of the separable iron with normal, or in comparison with this increase, relatively diminished values of the non-separable iron. Thus there results, especially at the beginning of the icterus, a definite increase of the total serum iron.

The abnormally increased *Heilmeyer* serum iron value in simple icterus was interpreted by *Hemmeler* as a sign of a disturbance of

the iron excretion via the bile ducts. This explanation is plausible, but it contradicts *Eppinger's* findings of normal iron excretion through the bile in catarrhal icterus. Our observations also showed that during icterus there was a normal or only slightly diminished iron content in the bile. Finally, the writings of *McCance* and *Widdowson* and of *Whipple* stress the fact that the possibilities in the healthy organism of effecting iron elimination, especially through the bile-ducts, are slight. We must therefore consider it unlikely that there is considerable reduction of the iron excretion in catarrhal icterus, since there is no indication that the increased serum iron content found here is due to such an obstruction in iron excretion through the bile.

The question now arises whether the three hypotheses mentioned above are reconcilable with the presence of an increase of the total iron. Added administration of ionised iron might explain an increase of the total iron; but the digestive disturbances would indicate as more probable a reduction of the intestinal iron absorption in catarrhal icterus. If there is a disturbance in the conversion of the separable into non-separable iron this does not indicate that the total iron is necessarily increased. We can still consider the possibility of a reduced consumption of the separable iron or of an inhibition in storing it in the depository organs of the body.

We are not in possession of any clinical data (pronounced anaemia, disturbances of basal metabolic rate, disturbances of tissue respiration, etc.) that would indicate an arrest of iron consumption in catarrhal icterus; on the contrary, it is easier to assume that the mechanism of storage of the separable iron might be disturbed. In the liver the iron is deposited in various forms. Now some injury to the hepatic cell, as seen in hepatitis in general and in catarrhal icterus in particular, might very well considerably impede or even completely paralyse the mechanism for storing the circulating iron in the hepatic parenchyma. As a result the iron would accumulate in the circulation and this would account for the increased values of the non-haemoglobin serum iron. Our observations permit us to go even further in our assumptions; for it is highly probable that the lesion in the hepatic cell not only prevents the deposition of iron in the liver, but also renders impossible the physiological conversion of the iron of the separable complexes.

This would explain the above-indicated increase of the total non-haemoglobin iron values of the circulating iron and the comparatively great increase of the A and B iron values. In mechanical icterus, due to the absence of a primary injury to the hepatic cell, this phenomenon would not present itself. On the

other hand, bile retention does not as a rule increase the serum iron value, for the capacity of excreting iron through the digestive tract is but slight, even in the healthy organism. In this way it would be possible to explain the different behaviour of iron metabolism in catarrhal and mechanical icterus.

These reflections arise from our observations of iron metabolism in catarrhal icterus in human beings. We have endeavoured to confirm them by experiment. By using phosphorus poisoning we produced more or less extensive hepatic injury in rabbits. Animals of the same age and sex, of a control group and a poisoned group, were injected intravenously. A few hours after the injection we determined simultaneously the total serum iron in the peripheral blood and in the blood of the hepatic vein, as well as the various iron fractions in the hepatic parenchyma. By this means, with the help of our analyses, we were able to determine whether intravenous administration increased the iron content of the liver and whether hepatic injury could affect the function of iron deposition in this organ.

Rabbit No. 1. Control animal without iron administration.

Total iron of serum from ear vein	..	280 γ%
Total iron of serum from hepatic vein	..	260 γ%

Iron of hepatic parenchyma	A-iron	10 γ%
	B-iron	450 γ%
	C-iron	25 γ%
	D-iron	0 γ%
	Total iron	475 γ%

Rabbit No. 2. Control animal with intravenous iron administration of 500 γ iron (3 hours after administration).

Total iron of serum from hepatic vein, 550 γ%

Iron of hepatic parenchyma	A-iron	10 γ%
	B-iron	600 γ%
	C-iron	80 γ%
	D-iron	40 γ%
	Total iron	730 γ%

Rabbit No. 3. Control animal with intravenous injection of 500 γ iron (3 hours after administration).

Total iron of serum from hepatic vein, 555 γ%

Iron of hepatic parenchyma	A-iron	0 γ%
	B-iron	580 γ%
	C-iron	80 γ%
	D-iron	40 γ%
	Total iron	700 γ%

Rabbit No. 4. Control animal with intravenous injection of 500 γ iron (6 hours after administration).

Total iron of serum from ear vein .. 500 γ%
Total iron of serum from hepatic vein .. 590 γ%

Iron of hepatic parenchyma	A-iron	15 γ%
	B-iron	735 γ%
	C-iron	150 γ%
	D-iron	—
	Total iron	900 γ%

Rabbit No. 5. Rabbit poisoned with phosphorus without intravenous administration of iron.

Total iron of serum from jugular vein .. 285 γ%
Total iron of serum from hepatic vein .. 300 γ%

Iron of hepatic parenchyma	A-iron	0 γ%
	B-iron	290 γ%
	C-iron	24 γ%
	D-iron	86 γ%
	Total iron	400 γ%

Rabbit No. 6. Rabbit poisoned with phosphorus, 3 hours after intravenous injection of 500 γ iron.

Total iron of serum from hepatic vein, 540 γ%

Iron of hepatic parenchyma	A-iron	10 γ%
	B-iron	250 γ%
	C-iron	140 γ%
	D-iron	160 γ%
	Total iron	560 γ%

Rabbit No. 7. Rabbit poisoned with phosphorus, 4 hours after administration of 500 γ iron.

Total iron of serum from hepatic vein, 760 γ%

Iron of hepatic parenchyma	A-iron	10 γ%
	B-iron	500 γ%
	C-iron	90 γ%
	D-iron	—
	Total iron	600 γ%

Rabbit No. 8. Rabbit poisoned with phosphorus, 6 hours after administration of 500 γ iron.

Total iron of serum from hepatic vein, 680 γ%

Iron of hepatic parenchyma	A-iron	10 γ%
	B-iron	300 γ%
	C-iron	150 γ%
	D-iron	50 γ%
	Total iron	510 γ%

Rabbit No. 9. Chronic phosphorus poisoning, 6 hours after administration intravenously of 500 γ iron.

Total iron of serum from hepatic vein, 710 γ%

Iron of hepatic parenchyma	A-iron	0 γ%
	B-iron	255 γ%
	C-iron	35 γ%
	D-iron	0 γ%
	Total iron	290 γ%

Rabbit No. 10. Chronic phosphorus poisoning, 6 hours after intravenous injection of 500 γ iron.

Iron of hepatic parenchyma	A-iron	0 γ%
	B-iron	320 γ%
	C-iron	50 γ%
	D-iron	100 γ%
	Total iron	470 γ%

The investigations which we have just described lead us to the following conclusions: After intravenous injection the iron in the hepatic parenchyma rapidly increases. But this increase is greatly reduced or even completely absent if the parenchyma is injured. Thus severe injury to the liver obviously prevents it from taking up iron after intravenous injection. This experiment therefore appears to confirm the hypothesis drawn from clinical observation and throws new light on the function of the liver in connection with iron metabolism.

The liver is, therefore, able to retain a certain quantity of circulating iron and may be considered a possible organ of storage and conversion in connection with iron metabolism. This would explain the fact that a hepatic lesion prevents the conversion of the separable iron into iron which is irreversibly bound to larger molecules or complexes. Finally, the regular presence of iron in the bile, as observed by us in all our cases (whether in human beings or various species of animals) leads us back to the concept of iron excretion through the bile-ducts. But the hypothesis of iron excretion through the liver does not agree with the conclusions drawn by numerous authors who regularly found very little iron excretion through the digestive tract. If, therefore, the continuous and regular excretion of iron through the bile, as noted by ourselves, is not accompanied by at least an equal excretion through the stools it must be assumed that the iron of the bile is reabsorbed through the intestine, is led back to the hepatic parenchyma, and re-enters the circulation.

We have endeavoured to solve this problem experimentally by using dogs.[1] The procedure of these experiments was as follows:

[1] We were able to conduct these experiments thanks to the courtesy of *Professor Dr. A. Fleisch,* Director of the Physiological Institute of the University of Lausanne.

We began by withdrawing blood from the jugular and portal veins of the anaesthetised animal. Next, heavy bile secretion was provoked by the administration of Decholin, while at the same time a considerable quantity of bile of a known iron content was introduced into the duodenum. Four hours, then again six hours, afterwards blood was drawn from the jugular and portal veins. The determination of the changes in the serum iron content in the blood of the portal vein, which occurred while considerable quantities of bile were flowing into the duodenum, served the purpose of informing us regarding the possible absorption of iron from the bile. In order to prevent the penetration of any dietary iron the animal was kept in a fasting condition for 24 hours. Finally we guarded ourselves against possible errors of interpretation resulting from any casual fluctuations of the serum iron during the experiment by comparing the iron values of the blood of the portal vein with those simultaneously determined of the iron from the peripheral blood (jugular vein).

There were certain sources of error which could not be avoided. The absorption of large quantities of bile rich in bile acid produced increased haemolysis which was especially noticeable in the sixth hour of the experiment. This haemolysis considerably hampered the estimations. However, we determined the serum haemoglobin content of the haemolysed blood by means of spectrophotometry, and were thus able to calculate the iron fraction attributable to the haemoglobin. On the other hand, we were not concerned with comparing the serum iron values before and after absorption of the bile, but with comparing the non-haemoglobin iron values of the peripheral blood circulation (jugular vein) with those of the blood coming from the intestine (portal vein). It was therefore possible to compare approximately

Iron values in plasma

	Before Introduction of bile	4 hrs later	6 hrs later
Blood from jugular vein			
A-iron	5 γ%	20 γ%	200 γ%
B-iron	165 γ%	140 γ%	140 γ%
C-iron	60 γ%	80 γ%	20 γ%
D-iron	40 γ%	60 γ%	30 γ%
Total iron ..	270 γ%	300 γ%	390 γ%
Blood from portal vein			
A-iron	0 γ%	10 γ%	120 γ%
B-iron	105 γ%	120 γ%	290 γ%
C-iron	80 γ%	130 γ%	50 γ%
D-iron	85 γ%	90 γ%	40 γ%
Total iron ..	270 γ%	350 γ%	500 γ%

The duodenal administration of bile was made with 300 cc. of bile, which in all contained 290 γ% of iron, sub-divided into the two following fractions: soluble in HCl = 200 γ% ; insoluble in HCl = 90 γ%.

the fluctations of the iron fractions of the blood of the greater circulation with those connected with the intestinal absorption without the danger of too great an interference from the haemolysis caused by the experiment.

The table on p. 182 shows the results of this experiment. The values listed under "total iron" correspond to the total non-haemoglobin iron of the plasma (i.e. after deduction of the haemoglobin iron calculated by the spectrophotometric determination of the haemoglobin in the haemolysed serum).

Four hours after the start of intestinal absorption we noted a definite increase of iron in the general circulation. On comparing the blood values of the greater circulation with those of the portal vein we observed a distinct iron increase in the blood which was in contact with the intestinal absorption. This augmentation was registered in the fractions of the separable iron, and appeared to be greatest six hours after the beginning of absorption. Although the experiment was not free from technical errors, our findings would indicate that there had been intestinal absorption of the bile iron, which in this way was brought back to the hepatic parenchyma.

This double circulation of the iron can well be compared, for instance, with that of the urobilin. It may be of practical significance for the organism. One part of the bile iron, considered as a product of decomposition, might become converted into the iron which is of biological use for the body, as a result of the co-operation of the duodenum and the intestinal absorption: it might be compared with the dietary iron and would be able to restart the cycle of serum iron metabolism.

Even though no great importance as regards its total amount can be attributed to iron excretion via the bile-ducts it may nevertheless be of some interest in connection with the iron conversion and its further utilisation in the organism. The quantitative determination of the iron in the bile shows that iron excretion through the bile-ducts is frequently dependent upon the condition of the hepatic parenchyma. This fact was already stressed by *Eppinger*, who was of the opinion that although the absolute excretion of the iron through the bile remained fairly constant in various hepatic diseases there might nevertheless be great variations in the relation between the excretion of bile and of iron from the liver. In normal individuals this value is 11–15 : 1; in haemolytic icterus, in haemochromatosis and in polycythemia the excretion of bilirubin is distinctly greater than that of iron. On the other hand, it is definitely lower as compared with that of iron in catarrhal icterus, in cirrhosis of the liver and in aplastic anaemia.

It is very possible that the loss of the bile iron (that is, the impossibility of reabsorbing it, as for instance can be seen in the course of a fistula of the gall-bladder) may in the long run constitute a definite deficiency, sufficient to provoke an anaemia with a lowered serum iron content. This anaemia would therefore indicate the by no means negligible significance of the absorption of the bile iron. Thus, in order to explain this anaemia, it would not be necessary to assume that the bile contained a substance needed for the erythropoietic regeneration (as *Leydermann* and *Tammann* have done) which would be formed during the destruction of the red blood corpuscles.

We have already mentioned the iron excretion through the bile as found in various types of animals; we here merely wish to draw attention to the role of iron excretion through the bile in the course of haemolysis.

First haemolysis

Iron in bile	Before haemolysis	At end of Phenyl-hydrazine treatment (10 days)	8 days later (Continuation of Phenyl-hydrazine treatment)
Bilirubinaemia ..	0.79 mg.%	2.16 mg.%	1.65 mg.%
Separable iron ..	14 γ%	150 γ%	90 γ%
Total iron	100 γ%	150 γ%	200 γ%

Second haemolysis

		Bilirubinaemia mg.%	A iron γ%	B iron γ%	C iron γ%	D iron γ%	Total iron γ%
Before Phenyl-hydrazine: Hb., 100%; Erythr., 6.9; Reticulocytes, 4%.	Serum	0.35	25	123	82	40	270
	Gastric juice		25	65			
	Bile of bile-ducts		12	54	69	5	140
	Bile of gall-bladder		18	128	24	30	200
On 5th day of Phenyl-h. treatment: Hb., 97%; Erythr., 6.1; Reticuloc., 7%.	Serum		60	130	40	60	290
	Gastric juice		50	150			
	Bile of bile-ducts		0	70	110	40	220
	Bile of gall-bladder		0	95	145	30	270
At end of Phenyl-h. treatment, 10th day: Hb., 87%; Erythr., 5.3; Reticuloc., 9%.	Serum	0.51	76	119	105	74	374
	Gastric juice		50	80			
	Bile of bile-ducts		0	85	100	25	210
	Bile of gall-bladder		0	210	20	20	250
15 days later: Hb., 87%; Erythr., 5.5; Reticuloc., 8%.	Serum	0.35	70	85	50	22	227
	Gastric juice		45	100			
	Bile of bile-ducts		10	130	20	0	160
	Bile of gall-bladder		20	135	25	5	185

In order to be able to determine with accuracy the various fractions of iron in the bile it was essential to avoid the intervention of the gastric juice, which also introduces iron into the duodenum and through the hydrochloric acid can modify the quality of the iron contained in the duodenal juice. We also separated the gastric juice from the duodenal juice by simultaneously washing out the stomach and duodenum. By maintaining permanent aspiration the gastric juice could be removed as soon as it was formed, without it having the chance of getting into contact with the bile.

On page 184 are the results of the analyses made on two separate occasions in an individual showing polycythaemia.

In one case of polycythaemia, which often showed augmented destruction of the red blood cells, there was a slight increase of iron excretion. But the serum iron, as a result of a greater consumption of this metal, was rather reduced, due to particularly active erythropoiesis. When we provoked haemolysis with the help of phenylhydrazine we noted a rise of the serum iron content with a simultaneous increase of the iron excretion through the bile and the gastric juice. This increase affected especially the closely bound and non-separable iron fractions. On the other hand, the easily separable iron of the bile appeared to diminish.

Here in conclusion are two cases of hepatic injury, one subacute, the other chronic, in which we determined the iron in the bile.

A. A., aged 42. *Hepatitis with enteritis.*

	In the serum	In the bile
A-iron ..	68 γ%	10 γ%
B-iron ..	92 γ%	14 γ%
C-iron ..	23 γ%	0 γ%
D-iron ..	100 γ%	26 γ%
Total iron ..	283 γ%	50 γ%

J. C., aged 61. *Cirrhosis of the liver.*

	In the serum	In the bile
A-iron ..	20 γ%	8 γ%
B-iron ..	140 γ%	68 γ%
C-iron ..	100 γ%	0 γ%
D-iron ..	75 γ%	60 γ%
Total iron ..	335 γ%	136 γ%

These two cases show a reduction, although only slight, of the iron excretion through the bile. Finally, it should be noted that in our experiments on rabbits poisoned with phosphorus an intravenous injection of iron was followed by a more considerable increase of the iron in the bile than was found in a normal individual. The explanation of this is as follows: A severe hepatic

injury has the effect that the liver can no longer retain in the parenchyma the iron which in part has escaped through the bile-ducts.

This concept might be confirmed by the study of the two following cases of severe cirrhosis:

B. L., aged 36. *Cirrhosis of the liver with splenomegaly, severe ascites, caput medusae.*

	Serum Bilirubin: 0.35 mg.%	
	Before the ascites puncture	6 days after the puncture
A-iron ..	0 γ%	0 γ%
B-iron ..	10 γ%	10 γ%
C-iron ..	315 γ%	325 γ%
D-iron ..	35 γ%	45 γ%
Total iron ..	360 γ%	380 γ%

M. E., aged 60, *Chronic cirrhosis, splenomegaly, ascites, caput medusae.* Great oedema of lower extremities.

	Serum Bilirubin: 0.2 mg.%	
	Before the ascites puncture	10 days after the puncture
A-iron ..	0 γ%	0 γ%
B-iron ..	130 γ%	135 γ%
C-iron ..	120 γ%	90 γ%
D-iron ..	90 γ%	85 γ%
Total iron ..	340 γ%	310 γ%

In the first case we noted a marked decrease of the easily separable iron.

In cirrhosis of the liver the mechanism of iron conversion appears to be more complicated. This case presented conjointly an injury of the hepatic cell and of the reticulum which leads to, in accordance with the extent and form of the cirrhosis, a preponderating disturbance either of storage and conversion or of excretion.

The liver therefore plays an important role in iron metabolism, both as an organ of deposit and one of conversion and excretion. We are tempted to view the excretion of iron through the bile as a process of conversion of this metal in preparation for its new biological utilisation in the organism. Hyperfunction of the hepatic reticulum, as observed in haemolysis, is accompanied by greater iron excretion in the bile. If iron is administered there may also be increased iron excretion through the bile-ducts.

especially if the injured hepatic cell is no longer capable of retaining the iron. On the other hand, a hepatic lesion is accompanied by reduction of the iron values in the bile if the hepatic parenchyma is unable to take up and retain the circulating iron. This would explain the "retention" of iron in the circulating blood in the course of catarrhal icterus.

Haemochromatosis is a problem of some interest in connection with hepatic iron metabolism. This disease is characterised by the presence of cirrhosis of the liver, insufficiency of the islet cells of the pancreas (diabetes mellitus) and a deposit of haemosiderin, haemofuchsin and melanin in the tissues. *Vannotti* had the opportunity of studying iron metabolism in a typical case of haemochromatosis. On several occasions he noted a definite increase of the serum iron with simultaneous increased excretion in the urine of urobilin, uroerythrin and porphyrin, as well as augmented bilirubinaemia. *Heilmeyer* also described an increase of serum iron in a case of haemochromatosis, whilst *Sachs, Levine* and *Griffith* found only normal values. In *Vannotti's* case the iron value rose slightly as the result of treatment with phenylhydrazine, this increase being less than that found in healthy individuals under the same conditions of experimentation. This reaction recalls the serum iron curve in haemolysis during experimental blockage of the reticulum, in which an increased serum iron content and slight increase of iron can often be observed during haemolysis. But histological investigation has shown that there is a fundamental difference between haemochromatosis and the blockage of the reticulum. Unlike haemochromatosis, during the blocking of the reticulum there is no definite increase of iron deposit in the tissues. Thus the analogies shown to exist between haemochromatosis and the blocking of the reticulo-endothelial system in serum iron metabolism do not extend to the functions of iron storage and excretion.

These observations lead to the assumption that in haemochromatosis iron excretion is arrested (*Fowler* and *Barer*, as well as *Marble* and *Smith*, observed great iron retention in four cases of haemochromatosis which were subjected to a strict iron balance), and as a result there is an increase of circulating non-haemoglobin iron and of deposit iron in the tissues. The question now arises: where is the seat of the lesion which impedes this iron excretion? The hepatic cell which is closely bound up with iron metabolism cannot be the main cause of this, otherwise there would be iron retention in all kinds of hepatic injuries, particularly in cirrhosis of the liver. Certain analogies noted in disturbances of iron metabolism, haemochromatosis and the

blocking of the reticulum might lead to the assumption that it was the Kupffer's cells and the reticulum as a whole which played the decisive aetiological role. But here also we found differences between our own case and that of experimental obstruction of the reticulum.

In haemochromatosis we are doubtless concerned with a combination of both mechanisms, i.e. with injury to the liver cell coexistent with a functional disturbance of the reticulo-endothelial system. The dysfunction of the reticulum would be constitutional in origin, as appears from the observations of various authors who recognise in the familial appearance of this disease a symptom of special constitutional significance.

To-day we know that there exists close co-operation between the hepatic cell and the reticulum, noticeable particularly in the conversion of blood pigment into bile pigment. As a result of the co-operation of the Kupffer's cells and the hepatic cells the porphyrin ring of the haemoglobin is opened up, in order to furnish the chain of the four pyrrole nuclei of the bilirubin; at the same time the iron released from the haemoglobin molecule is taken up. Now part of this iron is given off by the reticulum to the hepatic cell, which converts it or excretes it with the bile. It would appear that the transference of the iron from the Kupffer's cell to the hepatic cell is interrupted during haemochromatosis—a circumstance which would explain the increase of the non-haemoglobin iron in the blood circulation, both in the tissues and in the rest of the reticulum. On the other hand, the increased value of the serum iron would cause a progressive blocking of the reticulum, the clinical results of which would be a growing incapacity on the part of the bone-marrow to fulfil its function. In this manner it might be possible to explain the appearance, despite increased serum values, of hypochromic anaemia in severe cases of haemochromatosis.

This separation of the function of the Kupffer's cell from that of the hepatic cell would only be revealed by the appearance of a lesion of the hepatic parenchyma as a result of the influence of a toxic factor (alcohol, lead, infection, arsenic, copper, zinc, etc.).

We must also add concerning the subject of haemochromatosis the experiments of *Granick* and *Michaelis*, who observed in this disease that ferritin can be found abundantly in the liver and also in the duodenal mucosa and spleen. This fact indicates that haemochromatosis is not brought about by a failure to produce the specific protein, the apoferritin. Finally, *Cartwright* and *Wintrobe* found recently in a case of haemochromatosis 147γ% of non-haemoglobin iron.

VI. Iron and General Metabolic Disturbances

As we have already seen in the first part, iron plays an important role in connection with the control of cellular respiration, in consequence of its close connection with the mechanism of biological catalysis. The transportation of oxygen from the blood to the cell and its intracellular utilisation in the combustion of the nutrient substances, which represents the essential source of the organism's energy, is effected with the help of a system of catalysts, among which one of the leading parts is played by iron. The following are some of the catalysts and cell pigments which contain iron: Warburg's respiratory red enzyme, Cytochrome, Peroxydase, Katalase, etc. This dependence of cell respiration upon iron metabolism can be experimentally demonstrated, as is shown by the observations of *Kochmann* and *Seel* and also of *Douk* regarding the increase of cellular oxidation after iron administration.

We asked ourselves whether it would not be possible, in the case of normal and diseased persons, to draw indirect conclusions, based on an examination of iron metabolism, regarding the activity of these catalysts. The amount of iron needed for cell catalysis is very little. In the case of a normal individual it cannot be measured, since this minimal fraction contained in the total iron in circulation or entering into the tissues is too infinitesimal to be estimated or differentiated. Nevertheless it is conceivable that in certain cases where general metabolism has undergone great changes this iron might be clinically demonstrable. We have therefore endeavoured to indicate certain conditions under which the significance of the iron as a regulator of cell metabolism is revealed.

We made a special study in human physiology of the iron metabolism under the influence of muscular exertion, that is, at a time when the greatest demands are being made on the cellular oxidation of the muscle. This increased functioning of the cellular bio-catalysts is still more pronounced in high altitudes, where the reduction of the partial oxygen pressure demands greater activity on the part of the catalysts of cell respiration. *Vannotti* and *Markwalder* took up the examination of this problem and on the Jungfraujoch (at an elevation of 3450 m.) they studied the erythropoiesis, the non-haemoglobin iron content of the serum and blood, and the bilirubinaemia of different individuals who had been variously acclimatised to high altitudes, both during and after great muscular exertions. From this study we offer two observations which strike us as particularly instructive.

Date	Observations	Hb. %	Erythrocytes Mill.	Iron in blood γ%	Iron in serum γ%	Bilirubin mg.%
M. H., aged 23.						
Aug. 28.	In the valley ..	112	4.9	430	150	0.281
Sept. 1		112	4.3	550	150	0.323
Sept. 3.	Arrival at altitude of 3450 metres					
Sept. 4.	1 day later during rest	118	4.8	2870	480	0.665
Sept. 7.	During rest ..	120	5.1	480	210	0.238
Sept. 10.	After 2 days of very long and laborious mountaineering to 4000 m. (great exhaustion)	112	5.26	120	150	0.512
Sept. 16.	After good acclimatisation ..	126	5.9	720	180	0.385
Oct. 18.	1 month after return to valley ..	110	5.0	1080	170	0.431
V. A., aged 30. Individual thoroughly trained in mountaineering.						
July 30.	In the valley ..	100	4.75	630		0.387
Aug. 31.	After a first period of mountaineering	115	5.31	1300	334	0.531
Sept. 4.	12 hours after arrival at 3450 m.	120	5.52	900	223	0.538
Sept. 6.	After an ascent to 4000 m.	115	4.9	1030		
Sept. 10.	After 2 days of long and strenuous climbing ..	125	5.8	300	105	0.435
Sept. 11.		105	5.25	920		
Sept. 20.		120	5.17	920	125	0.396

These observations, as also a number of others published by *Vannotti* and *Markwalder*, have led us to the following conclusions: At high altitudes the number of red blood corpuscles increases, due to a mobilisation of the stored blood, particularly in the spleen. This increase is very transient and is often followed 12 to 24 hours after arrival in the mountains by a definite reduction of haemoglobin and of red blood cells. This haemolysis is also manifested by notable changes in the erythrocytes and by an increase of bilirubinaemia and of the circulating non-haemoglobin iron value.

The augmentation of the serum iron content is not of long duration; it recedes in the course of the succeeding blood regeneration, which is characterised by the appearance of larger and more resistant erythrocytes and by the disappearance from the circula-

tion of the iron needed for the formation of haemoglobin and erythrocytes. These erythropoietic changes are chiefly noticeable in untrained persons during repose and at high altitudes. But the condition becomes modified if this individual accomplishes great muscular feats.

The organism of the untrained individual reacts quickly to physical exertion by haemolysis, which is sometimes very considerable. The number of erythrocytes sinks very perceptibly and the bilirubinaemia increases. The value of the non-haemoglobin iron rises; but if the exertion is very great and of long duration it falls instead of rising, as might be assumed. It will even fall to exceptionally low values after particularly heavy exertions, and this reduction can sometimes even be noted in persons accustomed to high altitudes. We consider the following to be the probable explanation of this phenomenon: As an indication of a person's acclimatisation during the entire period of his stay in the mountains the organism will show very high values of circulating non-haemoglobin iron. This increased serum iron content of the acclimatised individual only gradually declines afterwards and does not completely disappear until one to three months after his return to the plains. These findings lead us to assume that in high altitudes the human organism needs considerable quantities of circulating iron, either for haematopoiesis or for muscular metabolism.

An organism in repose is supplied with the necessary quantities of iron by a preliminary haemolysis. The value of the circulating non-haemoglobin iron increases slowly; it is utilised in accordance with the peripheral needs during violent and continuous muscular exertions, during which time, as a result of accelerated cell respiration, a considerable addition of biologically active iron is required. The severe muscular work in the mountains mobilises great quantities of iron to the tissues, so that the serum iron content falls to very low values. If the quantities of iron in circulation and in the organs of storage are insufficient the body must resort to repeated haemolysis, in order to replace this iron. It mobilises iron from the red blood cells and can in this way balance its growing needs of this metal, indispensable both for the cellular chemical processes and for the muscular work. These regulatory processes operate in a less conspicuous manner in persons adapted to high altitudes, who possess a large supply of circulating iron reserves which have collected by a similar mechanism.

At our instigation *Merz* recently undertook a study of the problem of iron conversion in connection with muscular exertion. In the muscle of the rabbit he followed the values of iron, of cytochrome, of free and phosphorylated lactoflavin, of aneurin and of cocarboxylase under the influence of acute exertion and

muscular hypertrophy after repeated faradisation. In his experiments, by using a similar technique, *Merz* determined the four iron fractions of the serum in muscle and obtained the following results:

After one protracted acute muscular exertion the total iron usually increases, with distinct rise in the value of the iron fractions B and C; on the other hand, the fraction A often decreases. The myoglobin and cytochrome appear to be unaffected in this experimental group. Oxydase, vitamins B_2 and C are particularly affected by a severe muscular activity; the values of vitamin B_2 undergo but little change.

In chronic muscular activity, leading to muscular hypertrophy, the increase of the total iron, above all of the B and C fractions, is especially noticeable, and *Merz* recognises, as a parallel to this increase, an augmentation of the myoglobin and cytochrome content of the hypertrophied muscle. Oxydase also distinctly increases, as has been previously emphasised by *Guckelberger* and *Kaiser*. There is a special increase of lactoflavin in the hypertrophied muscle (often even twice as much as in the untrained musculature). The relation phosphorylated-lactoflavin: free-lactoflavin remains unchanged in both the trained and untrained muscle. The increase of vitamin B_2 (phosphorylated and free B_2) and of vitamin C is less pronounced.

These findings emphasise the particular significance of the cellular chemical processes in muscular exertion and throw a new light on the problem of the functional relationships existing between the enzymes and pigments of the tissues. Indeed *Merz* was able to determine, in addition to an increase of the iron-containing active substances of the cell (oxydase, myoglobin, cytochrome, etc.) an increase of the separable iron, as well as of the vitamins B_1 and B_2 acting as co-ferments in close co-operation with the iron.

These experiments thus afford us some data regarding the above-mentioned fluctuations of the serum iron during severe bodily exertions and show that the mobilisation of the actively functioning iron fractions is followed by an increase of the iron in the muscular tissue.

During our work in collaboration with *Delachaux* and *Tissières*, we could show the adaptation of iron metabolism to oxygen lack at high altitudes. In fact, at an altitude of 6,000–7,000 m., we observed a considerable increase of iron pigments in the body, but the increases are not parallel. In fact, we see that the haemoglobin increases from 30 to 50%, the myoglobin from 50 to 70% and the cytochrome C can increase from 100 to 200%. These differences are probably due to the

different biological actions of these three pigments. Finally, with the help of radio-active iron, we could see that this iron is almost entirely taken up in the bone-marrow and does not remain in the muscular tissues at high altitudes.

As a further supplement to what has been stated above, we record in the following table a number of results obtained by *Merz* in young persons who underwent great muscular exertions in the plains.

28 *Kilometre race* (in one stage).

Name and Constitution	Serum Iron At start γ%	Upon arrival γ%	24 hrs. after arrival γ%
(1) *St.* Aged 20. Small, thin, slight musculature, medium resistance during the effort	114	76	101
(2) *Fé.* Aged 20. Small, thin, slight musculature, very resistant ..	124	88	123
(3) *Va.* Aged 20. Short, great muscular development, resistant	94	90	111
(4) *Le.* Aged 20. Tall, average resistance, considerable fat deposit	98	96	126
(5) *Si.* Aged 20. Tall, thin, slight musculature, little resistance	90	94	108

85 *Kilometre race* (in 3 stages; total duration of run 52 hours).

Name and Constitution	Serum Iron At start γ%	Upon arrival γ%	24 and 48 hrs. after arrival γ%	
(1) *Co.* Aged 20. Tall, great muscular development. Indefatigable, very resistant	127	109	96	—
(2) *Br.* Aged 20. Perfect athlete, with average resistance	94	122	106	—
(3) *Ja.* Aged 20. Tall, abundant fat deposit, moderate musculature, slight resistance	77	111	115	105
(4) *He.* Aged 20. Tall, great muscular development, resistant	90	103	119	110

In the 28 kilometre race in one stage we noticed generally that after the exertion there was a distinct reduction of the serum iron content, with a tendency to a recovery of the normal value twenty-four hours after the exertion. In the case of two individuals showing little resistance and who were not adapted to great exertions there was no reduction of iron. We believe that in these cases the loss of circulating iron was compensated by iron mobilisation or by haemolysis, all the more so as twenty-four hours after the exertion the iron value was higher than at the beginning.

If in the course of a continuous exertion an individual was seen to be slightly resistant and showed symptoms of great fatigue (in the 85 kilometre race in three stages with a total duration of 52 hours), we saw, on the other hand, that upon arrival there was a definite increase of the iron value. After very probably having sunk at the beginning, the iron value soon rose as a result of utilisation and the neutralising haemolysis. Here we found a reaction approximately equal to that observed in the mountains.

In the domain of the infectious diseases we believe that we have found additional correlations between iron metabolism and the fundamental disturbances of cellular metabolism. Thus the definite reduction of the non-haemoglobin iron, as observed by various authors, particularly by *Heilmeyer,* occurring in acute and chronic infections and in febrile conditions, is not only to be attributed to a mobilisation of iron in the reticulo-endothelial system (a point we have already stressed in pages 79–151); rather, it is also due to the fact that the accelerated rhythm of the cell oxidations and the increased general metabolism, characteristic of fever, consume more iron in connection with more active chemical reactions of the cell. As has been emphasised above, this iron consumption of the cell may assume considerable importance in chronic infections, especially in pulmonary tuberculosis, in which condition a diminution of the pulmonary surface due to extensive lesions may bring about oxygen deficiency in the organism and cause heavy demands to be made upon the cellular biological catalysts, which have already become involved in consequence of the fever and the prophylaxis of the organism.

We give on page 195 the fluctuations of the various iron fractions in a case of severe fever provoked by an injection of Pyrifer.

We see, therefore, that while the temperature was rising the iron was visibly decreasing, and this applied equally to the total serum iron and to each of its fractions, more particularly to the easily split-off iron. The iron of the non-separable complexes appeared

D. M., aged 24 (see Diagram 9).

Remarks	A-iron γ%	B-iron γ%	C-iron γ%	D-iron γ%	Total iron γ%
Before injection	89	92	160	60	401
At beginning of high fever (38.4°)	33	57	180	30	300
5 hours later, towards end of first febrile attack (38.4°)	8	33	184	—	225
15 hours later. Meanwhile during several hours fever rose to 39° ..	33	39	50	60	182
5 days later	90	95	100	55	340

In a second case this Pyrifer injection caused only a slight rise in temperature, to 37.7°. The ionised iron did not change, the easily ionised iron diminished 20% during the febrile period; the total iron sank from 295 γ% to 110 γ%, rising 6 hours later to 226 γ%.

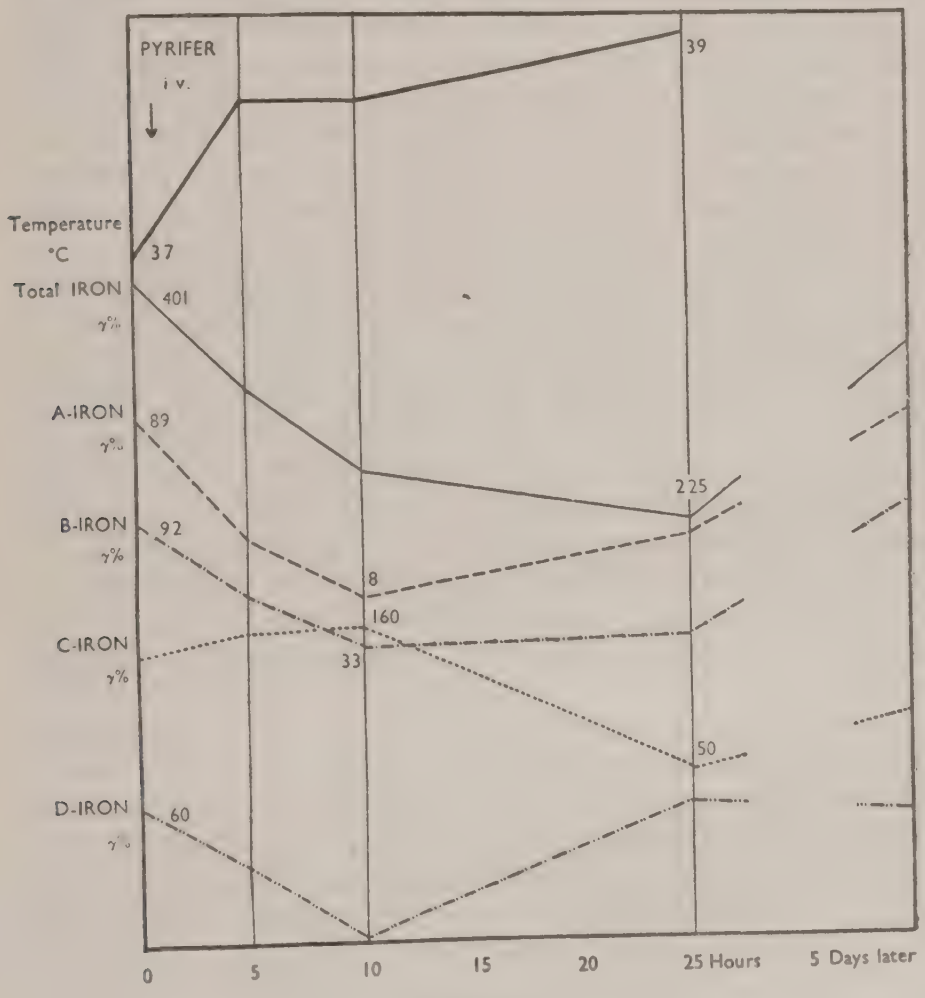

DIAGRAM 9

Fluctuations in the serum iron in the course of a severe febrile reaction (Pyrifer).

to possess the greatest stability. The decline during the febrile period of the iron fraction which can be precipitated with the protein thus indicated an extensive change of the blood proteins. The reduction of the serum iron content was noticeable not only during the period of fever but also some hours later. Next A- and B-irons gradually increased and this was followed by a progressive rise of D-iron. The balance was only established several days after the outbreak of the fever.

All these observations lead us to assume that it is primarily the iron fractions of the separable complexes, i.e., the fractions which most often take part in the iron conversion, which show the greatest variations. These fractions represent, in part at least, the active iron of *Starkenstein.* But since the same fractions also show the greatest quantitative changes in the course of haemolysis and of new blood formation, we must conclude that the intensity of the exchanges incident to iron metabolism are chiefly associated with the latter. In this way we can explain the frequent occurrence of hypochromic anaemias with lowered serum iron content in the course of infectious diseases or of protracted febrile conditions. The mobilisation of iron for the needs of cell metabolism and for the stimulation of the reticulum diminishes the serum iron content; it affects primarily the iron fractions A and B, which are most necessary for the normal functioning of the bone-marrow.

This added need of iron on the part of the organism in infections and inflammatory conditions is clinically revealed, not only in anaemia (i.e. a form based not only on iron deficiency, but also upon a toxic inhibition of the bone-marrow activity, which is anatomically manifested in an enrichment of the tissues and reticulum in iron); it is also seen in the good results attained from systematic iron therapy in chronic infectious diseases. At the same time as *Heilmeyer,* we ourselves noted a distinct improvement, not only of the general condition, but also of the basic disease, in a number of chronic febrile infections, such as rheumatism, pulmonary abscess, osteomyelitis, and above all, pulmonary tuberculosis.

More acute inflammatory processes, such as pneumonia and severe furunculas or whitlows, will sometimes also react exceptionally well to intravenous injections of iron. The method of oral iron administration, which is so successful and often superior to intravenous iron therapy, in the treatment of certain forms of anaemia, is, according to our own experience, less effective in the above-mentioned infectious conditions, especially if they assume an acute form.

Finally, we mention a few additional observations in connection with iron metabolism in cachexia. In this condition the

organism is in a state of reduced tissue and cellular activity, either as a result of an involution of the various vital functions associated with the tissues and the organs, or due to a great consumption of the most important products of cellular synthesis. Here are three cases of cachexia of varying aetiology: senility, chronic infection, and neoplasm.

Name	Age	Condition	A-iron γ%	B-iron γ%	C-iron γ%	D-iron γ%	Total iron γ%
H.D.G.,	aged 81	Senile cachexia, generalised arterio - sclerosis	8	112	130	44	294
Fr.D.L.,	aged 65	Toxic - infectious cachexia	10	110	50	60	230
Fr.B.M.,	aged 75	Severe cachexia after gastric carcinoma with extensive metastases	10	110	75	70	265

These three cases show a slight reduction of the various iron fractions. This might be explained by a reduction of the body's iron needs during the organic involution or by a consumption of iron in the case of the chronic infection and the tumour growth. This deficiency, due to various aetiological causes, affects chiefly the separable iron, which is entrusted with the most important functional role; yet it does not spare the other fractions, nor the total iron.

We extended our control investigations to yet another domain of the disturbances of general metabolism, i.e. to the conditions of conspicuous cyanosis which are the result of severe insufficiency either of circulation or of respiration (severe attacks of asthma or destruction of the pulmonary parenchyma, etc.).

After a severe pulmonary or circulatory disturbance the exchange of gases in the lungs and the periphery can be effected in only an imperfect manner and may lead to oxygen deficiency. We asked ourselves whether in such cases the organism might be made to react by inducing it to mobilise biologically active iron which, by increasing its catalytic activity, might help to maintain the endangered cellular gas exchange.

Here is a case of renal hypertension with chronic circulatory insufficiency and attacks of pulmonary oedema:

C. E., aged 48.

Date	Condition	Hb. %	Erythrocytes Mill.	Serum bilirubin mg.%	A-iron γ%	B-iron γ%
Aug. 10.	First acute attack. Severe cyanosis + difficulty in breathing ..	77	4.4	0.68	0	47
Aug. 14.	4 days after attack. No cyanosis. Slight difficulty in breathing	77	4.4	0.52	10	38
Aug. 18.	After an exertion, new violent attack of difficulty in breathing and severe cyanosis .. Venesection rapidly improved condition of patient, but on	76	4.3	0.49	0	80
Aug. 25.	Fresh signs of severe circulatory insufficiency, with increase of A-iron to 145 γ%.					

We were permitted to make a more complete observation of a patient 46 years of age, Mrs. P. M., suffering from severe mitral-stenosis (rheumatic endocarditis). The circulatory disturbances became exacerbated, especially during her first pregnancy at the age of 28. From that time on slow increase of cardiaic insufficiency with appearance of an auricular flutter with cyanosis, and congestion of the lungs and liver. Latterly onset of pronounced cyanosis with attacks of pulmonary oedema.

July 25. Pulmonary oedema.
Hb., 67%. Erythrocytes, 4,900,000. Serum-bilirubin, 0.66 mg.%. A-iron = 0 γ%. B-iron = 107 γ%.
Sept. 18. New attack of pulmonary oedema.
Hb., 71%. Erythrocytes, 4,900,000. Serum-bilirubin, 0.64 mg.%. A-iron = 54 γ%. B-iron = 83 γ%.
Sept. 19. Definite subjective and objective improvement after administration of analeptics and inhalation of oxygen for 30 minutes.
A-iron = 37 γ%. B-iron = 70 γ%.
On same day new violent attack. During crisis: A-iron = 53 γ%. B-iron = 84 γ%.
Sept. 21. After inhalation of oxygen and therapeutic circulatory stimulation, marked improvement.
A-iron = 36 γ%. B-iron = 68 γ%. But on
Sept. 22. Renewed attack after exertion, marked cyanosis.
A-iron = 44 γ%. B-iron = 85 γ%.
A venesection improves condition of patient.
Sept. 23. A-iron = 35 γ%. B-iron = 48 γ%.
Sept. 30. Very severe attack with heavy pulmonary oedema.
A-iron = 38 γ%. B-iron = 87 γ%.

A few months later we were able to determine the total iron and its four fractions in this same patient.

Remarks	A-iron γ%	B-iron γ%	C-iron γ%	D-iron γ%	Total iron γ%
During a severe attack of breathing difficulty and cyanosis	65	120	15	40	240
3 days later after stimulation and oxygen inhalation	40	100	80	40	260
1 week later renewed cyanosis with severe circulatory insufficiency	20	130	50	75	275
After intensive treatment, good improvement on 7th day of treatment ..	0	130	40	100	270
1 week later, the favourable general and circulatory conditions continued ..	0	90	80	100	270

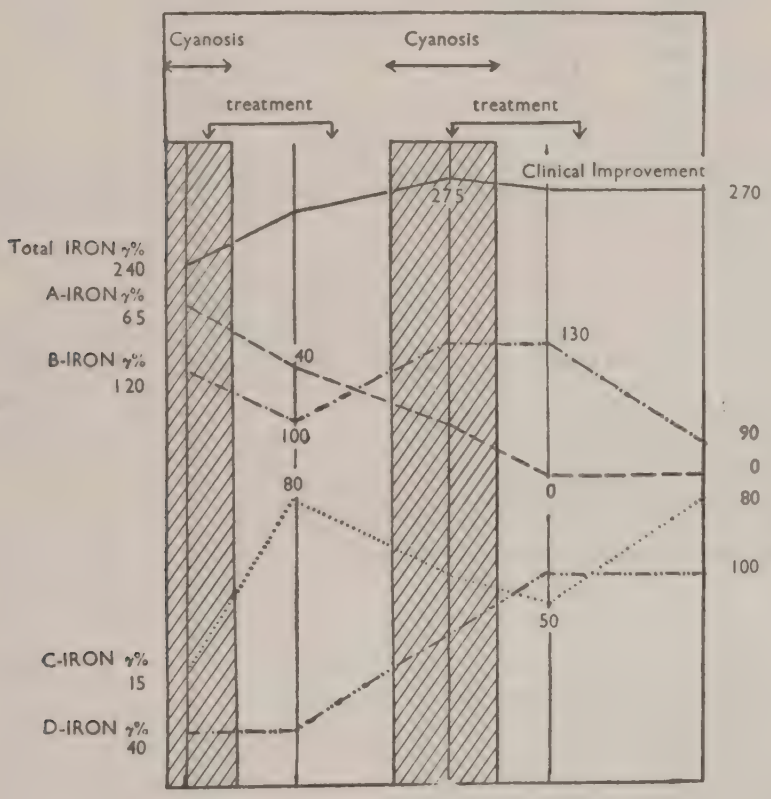

DIAGRAM 10

Serum iron fluctuations in a case of severe cardiac insufficiency with attacks of difficulty in breathing.

Here are a few cases of cyanosis produced by extensive destruction of the pulmonary parenchyma or by severe attacks of asthma.

(1) B. C., aged 51. *Severe bronchial asthma.*

Condition	Eosinophilia in blood %	Serum bilirubin mg.%	A-iron γ%	B-iron γ%
During the attack	10	0.61	10	100
Rp. Asthmolysin; 2 hours later disappearance of asthma attacks and cyanosis	6	0.56	0	100
2 days later, renewed attack ..	15	0.63	20	198
5 days later the attack has completely passed	17	0.67	40	70

(2) C. M., aged 33. *Continuing cyanosis caused by extensive sarcoidosis of the lung.*

	Before treatment	After one day's rest in bed, during which therapeutic circulatory stimuli and oxygen inhalations were given
A-iron	8 γ%	8 γ%
B-iron	104 γ%	92 γ%
C-iron	58 γ%	70 γ%
D-iron	70 γ%	65 γ%
Total iron	240 γ%	235 γ%

(3) The following case is one of extensive cirrhotic pulmonary tuberculosis, with pulmonary emphysema and congestion in the pulmonary circulation.

Date	Condition	Erythrocytes Mill.	Serum bilirubin mg.%	Blood reduction mm.	A-iron γ%	B-iron γ%
May 29.	Condition relatively compensated. Hb. 102%	5.7	0.58	12	0	100
July 22.	Period of increased pulmonary congestion, dyspnoea and cyanosis				42	175
July 28.	Temporary improvement after a period in bed and treatment with analeptics				8	110
Aug. 10.	Intense coughing with considerable expectoration ..		0.50		32	120
Aug. 24.	Pulmonary infiltration with temp. up to 38°C.			43 (during 1st hour)	10	200
Sept. 1.	Improvement. Temp. normal.			51	10	80
Oct. 26.	Cardiac disturbances. Pulmonary condition stationary. Cyanosis and breathing difficulty			18	30	280
Nov. 4.	Improvement of circulatory condition				20	80

(4) J. C., aged 35. *Bilateral pulmonary tuberculosis*; marked infiltration on left, treated by pneumothorax. Vital capacity before pneumothorax = 3260 cc.; 2 months after induction of pneumothorax = 1700 cc.; 3 months later = 2260 cc.

Condition	Temp. °C.	Blood red'n. mm.	Hb. %	Erythrocytes Mill.	Serum iron A-iron γ%	B-iron γ%	Pulse
5 days before refill of pneumothorax ..	37.0	12	92	5.3	21	75	72
24 hours after refill (breathing difficulty)	36.6	8	94	5.4	17	105	84
24 hours after a particularly large refill. Great breathing difficulty	36.6	8	99	5.1	21	160	87
10 days later. Slight difficulty in breathing	36.7	14	99	5.1	10	75	84
10 days later	36.9	12	99	5.1	21	110	80

(5) M. J., aged 36. *Bilateral diffuse pulmonary tuberculosis.* Cavity on right. Tendency to cicatrisation on left. Very extensive lesions with difficulty in breathing at the slightest exertion.

Temp. 36.9°C. Blood sedimentation 16 mm. Pulse 88. A-iron 25 γ%. B-iron 220 γ%.

We have described other similar cases on pp. 145–150.

From the above observations, which all appear to agree, we are able to recognise the presence during severe cyanosis of a certain, if not a considerable, increase of the non-haemoglobin iron and particularly of the separable iron, especially where the inadequate exchange of gases in the lungs and at the periphery is due to insufficiency in the pulmonary circulation. This rise in the serum iron value often declines if, due to the operation of a cardiac stimulus or oxygen inhalation, the cyanosis quickly vanishes. But it is less noticeable or even totally absent if the cyanosis becomes chronic. It appears of interest to note at this point that as early as 1931 *Langer* described a definite increase of the serum iron (320 γ%) in five cases of laborious breathing (asthma).

If the cyanosis is chiefly caused by pulmonary destruction the increase in the serum iron is less distinct; but it can always be observed in certain cases where it is accompanied by disturbances of the circulation. In our patient No. 3 the separable iron diminished at the time that the evolutive pulmonary tuberculosis broke out, even while the cyanosis and the breathing difficulty persisted. In the next attack of cyanosis, which was provoked by a disturbance of circulation and not by a process of pulmonary infiltration, the iron definitely increased again.

Finally, it might be concluded that the increase of the circulating non-haemoglobin iron in cyanosis is due to increased blood

destruction occurring during the circulatory insufficiency. But this view does not strike us as very plausible for the following reasons: We have found that during cyanosis the serum iron content does not increase in proportion to the bilirubinaemia. The latter increases in only a few cases in which the hepatic stasis has attained a certain intensity; but it cannot be ascribed to augmented haemolysis, all the more so as the total iron is also not increased, as would be the case if there were destruction of red blood corpuscles.

This last point must be particularly stressed. Here there is no increase of the total circulating non-haemoglobin iron, but rather a qualitative alteration in the distribution of the four iron fractions, manifested in an increase of the iron of the separable complexes at the expense of the metal which is irreversibly bound to large molecules or to protein. Thus there is a mobilisation of biologically active iron, according to the definition of *Starkenstein,* which is indispensable for the maintenance of the respiratory function of the blood in an organism impoverished in oxygen as a result of the inadequacy of the pulmonary exchanges of gases.

This represents a phenomenon similar to that seen after severe bodily exertion, especially in high altitudes, as previously described. In this last case it is the peripheral cell (muscular tissue) which needs iron possessing catalytic functional power in order that it may support very active chemical processes. In cyanosis, on the other hand, iron mobilisation is the consequence of an insufficient exchange of gases in the lung. Both processes provoke a transfer of the biologically active iron towards the periphery, with a corresponding reduction of the circulating iron. The first of these two different situations results from a lack of oxygen, the second from an increased need of oxygen; both are associated with the mechanism of the mobilisation of iron possessing a biocatalytic effect (separable iron), which is either called to the periphery or left in the circulating blood, according to the circumstances.

We now come to the discussion of the conversion of iron in the course of another form of cyanosis, i.e., that observed in connection with the treatment by large dosages of sulphanilamide.

In the course of our investigations on iron our attention was drawn to the problem of the effect of sulphanilamide; we accordingly undertook a series of experiments about which we wish to report in brief.

The mechanism of action of these substances is still not clarified. We know that the sulphonamides do not act on bacterial cultures; they accelerate neither the tissue resistance to the microbial invasion nor the local inflammatory reaction of the organism. Rather it would appear that the effect of the sulphonamides on the

microbes is not that of a disinfectant, but rather of a specific check to their virulence (antibiotic); accordingly it is easy for the body to eliminate and destroy them by inflammatory reaction and with the help of its various mechanisms of defence.

Certain authors have indicated that it would be by no means inconceivable that the sulphonamides, in addition to their displacing action on para-aminobenzoic acid, act similarly to certain redox systems and by means of oxidation of the free amino group cause the formation of a hydroxylamino group which might possibly be toxic for the microbes. Although this hypothesis has been greatly discussed and criticised it nevertheless merits our attention, especially since we now know that the sulphonamides are able to provoke a conversion of haemoglobin into methaemoglobin—a fact which would explain the occurrence of the special cyanosis during the administration of considerable doses of sulphanilamide and might represent the manifestation of hydroxylamine formation.

According to this view it would have to be assumed that the sulphanilamides have a certain influence on the system of cell respiration, both of the microbes and of the host. In order to enter more closely into this question we first studied the fluctuations of the serum iron during sulphanilamide treatment and next the iron metabolism in yeast cultures previously treated with these substances. At the same time the various important pigments and enzymes connected with respiration were also determined quantitatively (*Crausaz* and *Delachaux*). Here are a few examples:

L. A., aged 24. *Gonorrhea*: before treatment.

Hb. %	Erythrocytes Mill.	Serum bilirubin mg.%	Urobilin elimination in urine mg.	Porphyrin elimination in urine
97	4.89	0.4	3.00	Traces
		A-iron	10 γ%	
		B-iron	240 γ%	
		C-iron	110 γ%	
		D-iron	15 γ%	
		Total iron	375 γ%	
On the third day of treatment: 8.5 g. of sulphanilamide daily.				
85%	5.07	0.4	0.306	4 mg.
		A-iron	5 γ%	
		B-iron	335 γ%	
		C-iron	80 γ%	
		D-iron	10 γ%	
		Total iron	420 γ%	

P. M., aged 30. *Gonorrhea.*

Hb. %	Erythrocytes Mill.	Reticulocytes %	Serum bilirubin mg. %	Urobilin elimination in urine mg.	Porphyrin elimination in urine
Before the treatment:					
72	4.18	3	0.5	2.2	Traces
			A-iron 0 γ%		
			B-iron 180 γ%		
			C-iron 30 γ%		
			D-iron 60 γ%		
			Total iron 270 γ%		
2 days after the treatment: 7.5 g. of sulphanilamide daily.					
72	4.2	5	0.5	0.81	Traces
On 6th day of treatment: After 37.5 g. of sulphanilamide:					
70	3.9	10	0.4	Traces	2.25 mg.
			A-iron 0 γ%		
			B-iron 200 γ%		
			C-iron 10 γ%		
			D-iron 75 γ%		
			Total iron 285 γ%		
4 days after end of sulphanilamide treatment:					
87	4.3	21			

H. A., aged 47. *Lobar pneumonia on left side.*

On second day after onset of pneumonia: Fever 39.9°C.

Hb. %	Erythrocytes Mill.	Leucocytes	Reticulocytes %	Serum bilirubin mg. %	Urobilin elimination in urine mg.	Porphyrin elimination in urine
83	4.64	19,400	1	0.5	1.34	Traces
			A-iron	0 γ%		
			B-iron	74 γ%		
			C-iron	51 γ%		
			D-iron	5 γ%		
			Total iron	130 γ%		

On following day (after 10.5 g. of sulphanilamide) fall in temp. The urobilin elimination increased greatly to 10.35 mg.

Hb. %	Erythrocytes Mill.	Leucocytes	Reticulocytes %	Serum bilirubin mg. %	Urobilin elimination in urine mg.	Porphyrin elimination in urine
At the beginning of the third day of treatment, after 20 g. of sulphanilamide:						
75	3.9	10,000	6	0.15	2.59	1.81 mg.
			A-iron	0 γ%		
			B-iron	210 γ%		
			C-iron	60 γ%		
			D-iron	7 γ%		
			Total iron	277 γ%		
On 6th day of treatment, after 46.5 g. of sulphanilamide.						
74	4.36	8,300	6	0.25	2.23	3.18 mg.
			A-iron	0 γ%		
			B-iron	260 γ%		
			C-iron	50 γ%		
			D-iron	35 γ%		
			Total iron	345 γ%		
2 days after end of sulphanilamide treatment.						
82	4.36	11,000	23	0.25	1.36	Traces
			A-iron	0 γ%		
			B-iron	175 γ%		
			C-iron	40 γ%		
			D-iron	25 γ%		
			Total iron	240 γ%		
9 days after end of sulphanilamide treatment.						
88	4.09	6,100	6	0.15	1.23	Traces
			A-iron	0 γ%		
			B-iron	190 γ%		
			C-iron	30 γ%		
			D-iron	20 γ%		
			Total iron	240 γ%		

In addition to the case of pneumonia we purposely examined several cases of gonorrheal inflammations, which have the advantage of representing a not too severe infection, accompanied by involvement of the whole organism but devoid of a severe pulmonary injury with its accompanying disturbance of gas exchange.

These few examples selected from a number of systematic investigations have enabled us to reach the following conclusion: During the sulphanilamide treatment there is a very slight reduction of the haemoglobin content and of the number of erythrocytes in the blood. This condition, which is not necessarily dependent upon

the basic disease (we can also see it in the comparatively mild cases of gonorrhea), can either be provoked by increased blood decomposition due to sulphanilamide, or by a check in bone-marrow regeneration during the treatment. The first hypothesis might be supported by the formation of methaemoglobin, which predisposes to increased haemolysis. Moreover, the investigations of the iron metabolism would tend to strengthen this view. Actually, despite the existence of inflammation, we regularly noted that soon after the start of the treatment the iron values rose. This increase, however, was especially noticeable in the B-iron, i.e. the separable iron; the D-iron, which usually increases greatly during haemolysis, remained unchanged.

At all events the findings of the iron investigation do not uniformly support the view that there is increased blood destruction in sulphanilamide treatment. Moreover, an additional argument against this view is offered by the fact that we were unable to observe either increased serum bilirubin or severe bilirubinuria after the administration of sulphanilamide. *Rimington* thinks that methaemoglobin is transformed directly into porphyrin. Lastly, it is interesting to note that in a case of idiopathic methaemoglobinaemia, we found an increase of non-haemoglobin iron to between 250–300 $\gamma\%$.

If, therefore, certain symptoms are strongly indicative of an increase of blood destruction under sulphanilamide treatment, nevertheless, a more exact analysis of our cases would make it appear rather more probable that the sulphanilamide haemolysis must be very slight. It seems that the condition in question is rather a disturbance of the iron porphyrin complex that we are accustomed to observe in injury to the cellular regulation of respiration.

As soon as the effect of the sulphanilamide ceases, both the iron and porphyrin stop increasing and the pathological porphyrinuria vanishes, whilst the blood picture, as a result of the compensation augmentation of erythropoiesis (reticulocyte increase) rapidly returns to its initial values. This last finding supports the view that the occurrence of slight anaemia during the administration of these bodies is due to an arrest of the functioning of the bone-marrow.

In support of the theory gained by clinical material that these drugs fundamentally affect the functioning of the tissues, *Crausaz* endeavoured to obtain some insight into the mechanism of tissue respiration in yeast cultures. He came to the following conclusion: The addition of sulphanilamide to yeast cultures causes a definite reduction in the concentration of oxydase, catalase and cytochrome. The vitamin B_1 content is diminished, while that of vitamin B_2 remains unchanged. The iron content of the cell shows similar relations as in human serum, i.e. a

definite increase of B-iron (separable iron) up to 50–70% of the initial values; but the C-iron only increases slightly. In brewer's yeast the porphyrins definitely increase, but in baker's yeast they rather tend to diminish. These findings naturally vary in accordance with the concentration of the chemical structure of the sulphanilamide preparation and the type of yeast. But they give us a fairly clear indication of the mechanism of the sulphanilamide effect on the respiration of the cell.

The aerobic phase of the yeast activity appears to be somewhat hampered by the sulphanilamide. The iron-porphyrin complexes which we have determined (oxydase, catalase and cytochrome) are reduced, while at the same time the iron and porphyrin rise, and this explains that the sulphonamides very probably exert an arresting or disturbing effect on the system. On the other hand, the aerobic enzymatic phase does not appear to be greatly influenced.

The enzymes dependent upon the vitamins of the B-group are probably not greatly changed; this accounts for the fact that elimination of vitamins B_1 and B_2 did not appear to be particularly disturbed in the individuals we observed. All these findings confirm, to a certain extent, the view based on the investigations of iron metabolism in human subjects, that sulphanilamide exerts a profound effect on the system of cellular respiration, and that for this both the tissue iron and its porphyrin carrier are mobilised. In the case of the bacteria in which the mechanism of respiration is less developed and differentiated the partial inhibition of certain cellular catalysts, in association with the displacement of substances which promote growth, might have the effect of paralysing their toxic activity.

We now come to the discussion of a final problem of interest in connection with iron metabolism, i.e. that of the relationship existing between non-haemoglobin iron and the disturbances of basal metabolism, as seen in pathological thyroid activity. The investigations of certain authors (*Locke, Main* and *Rosbach, Moore, Doan* and *Arrowsmith, Skouge, Vahlquist*) show for the most part that in thyroid diseases there is a normal serum iron content, even where there is anaemia. We have followed the curves of the various iron fractions in hypothyroidism during thyroid treatment and also in hyperthyroidism.

Here are a few cases of hypothyroidism with myxoedema:

F. C. E. Before treatment:
Basal metabolism: −16.1, −21.8.
Cholesterin: 370 mg.%. Weight, 47.2 kg.

1st determination: A-iron=28 γ%. B-iron=73 γ%.

2nd determination: A-iron=38 γ%. B-iron=70 γ%.

During treatment with thyroid extracts:
A-iron=28 γ%. B-iron=80 γ%.

At end of treatment: Cholesterin, 280 mg.%.
A-iron = 20 γ%. B-iron = 19 γ%.
2 weeks later: Weight, 46 kg. Cholesterin, 170 mg.%.
A-iron = 5 γ%. B-iron = 30 γ%.

Mr. R. E., aged 61. *Hypothyroidism with Myxoedema. Deforming Arthropathy.*

Before treatment: Barkan iron, 132 γ%. Heilmeyer iron, 165 γ%.

After 3 weeks' treatment: Reduction of weight, 2 kg.
Barkan iron, 28 γ%. Heilmeyer iron, 115 γ%.

1 week after end of treatment:
Barkan iron, 14 γ%. Heilmeyer iron, 24 γ%.

2 weeks after end of treatment:
Barkan iron, 32 γ%. Heilmeyer iron, 152 γ%.
New reduction of weight of 2 kg.

F. P. M. *Marked Hypothyroidism.*

		1st determination	2nd determination
Before treatment:	Barkan iron ..	5 γ%	7 γ%
	Heilmeyer iron ..	20 γ%	18 γ%
	Cholesterin ..	210 mg.%	

After 6 days of thyroid treatment:
Barkan iron, 28 γ%. Heilmeyer iron, 72 γ%. Cholesterin, 170 mg.%.

8 days later:
Barkan iron, 42 γ%. Heilmeyer iron, 179 γ%.

1 month later:
Barkan iron, 7 γ%. Heilmeyer iron, 32 γ%. Cholesterin, 190 mg.%

H. P. A., aged 47. *Typical Myxoedema.*

Before treatment:
Basal metabolism −41.5%, −46.6%. Weight, 84.7 kg.
Cholesterin, 370 mg.%. Pulse, 60.
Barkan iron, 20 γ%. Heilmeyer iron, 65 γ%.

A beginning was made with a treatment of thyroid extract in small doses (0.4 g. thyroid powder daily).

After 8 days:
Pulse, 64. Cholesterin, 300 mg.%.
Barkan iron, 8 γ%. Heilmeyer iron, 96 γ%.

The daily dosage was raised to 1 g. Good subjective improvement:
Pulse, 90. Cholesterin, 140 mg.%. Weight, 83.3 kg.
Barkan iron, 12 γ%. Heilmeyer iron, 145 γ%.

The treatment was continued for 3 weeks with the same doses. The patient became accustomed to the treatment:
Pulse, 64. Cholesterin, 180 mg.%. Basal metabolism, −34.2, −37.

The iron gradually diminished again:
Barkan iron, 8 γ%. Heilmeyer iron, 55 γ% to 28 γ%.

As the result of ample administration of thyroid:
Weight, 82 kg. Pulse, 92.
Barkan iron, 20 γ%. Heilmeyer iron, 140 γ%.

These cases do not permit us to draw any definite conclusions, since thyroid treatment is sometimes accompanied by a reduction of the serum iron values. Nevertheless it would appear that on the whole in hypothyroidism the values of the circulating non-

haemoglobin iron tend to be low. (*Cartwright* and others found recently in three cases of hypothyroidism the following values for serum iron: 77γ%, 75γ% and 157γ%). With the help of our fractional method of determination we were able to follow the iron metabolism accurately in a typical case of myxoedema in a man of 48 (see Diagram 11).

The following are the results of our analyses:

	Before treatment	Treatment 0·6 g. Thyroid powder + 1 mg. of Thyroxine intramuscularly						The patient resumed his work with continued thyroid treatment		
Date	12, III	20, III	26, III	3, IV	21, IV	2, V	9, V	16, V	31, V	16, VI
Weight	Kg. 82·400	80·500	77·700	77·200	77·500	77·700	78·200	—	77·500	75·700
Basal met'm ...	−36·2%	—	—	−18·3%	—	−13%	—	—	—	−18·2%
Haemoglobin...	83%	75%	70%	70%	77%	—	77%	—	82%	76%
Erythrocytes (Mill.) ...	4·3	3·7	3·5	4·0	4·5	—	4·0	—	4·0	4·0
Cholesterin mg.%	340	215	150	165	—	190	—	200	—	190
	γ%	γ%	γ%	γ%	γ%	γ%	γ%	γ%	γ%	γ%
A-iron	15	98	15	10	25	10	10	10	10	10
B-iron	75	132	150	110	85	245	155	145	105	105
C-iron	30	0	65	70	60	0	10	65	125	95
D-iron	20	60	30	100	110	105	135	90	80	80
Total iron ...	140	290	260	290	380	360	310	310	320	290

In this special case we determined clinically a prompt improvement of the general condition with a rapid decline of weight, considerable reduction of cholesterin and increase of the basal metabolism during the first 15 days of treatment. On the other hand, the haemoglobin value and the number of red blood cells diminished. After the initial improvement the general condition became stabilised during the following days, the weight remained stationary, as did also the cholesterin value. The basal metabolism continued to rise, as well as the haemoglobin and the erythrocytes, which gradually attained their normal value. When, finally, the marked improvement permitted the patient to resume his work we again noted a reduction of weight, another slight diminution of the blood values (tendency to anaemia) and a certain decline of the basal metabolism.

The observation of this case thus showed the four different phases:

Phase 1: Severe hypothyroidism before treatment.
Phase 2: Rapid improvement of the general condition at the start of treatment.
Phase 3: Stabilisation of the improved condition.
Phase 4: Physical work after improvement.

To these four phases there corresponded four phases of the iron metabolism. During the first stage prior to treatment all the iron values were very low; in the second, during the rapid clinical improvement, a prompt and decided increase of the separable iron, accompanied by some rise of the total iron, could be registered. In the third stage of stabilisation, the A- and B-iron values

fell, whilst the total iron increased considerably. The patient was given thyroxine in order to accelerate the process of recovery. Next, a new definite increase of the separable iron was observed. During the fourth stage, the period of muscular work, all the iron values definitely diminished.

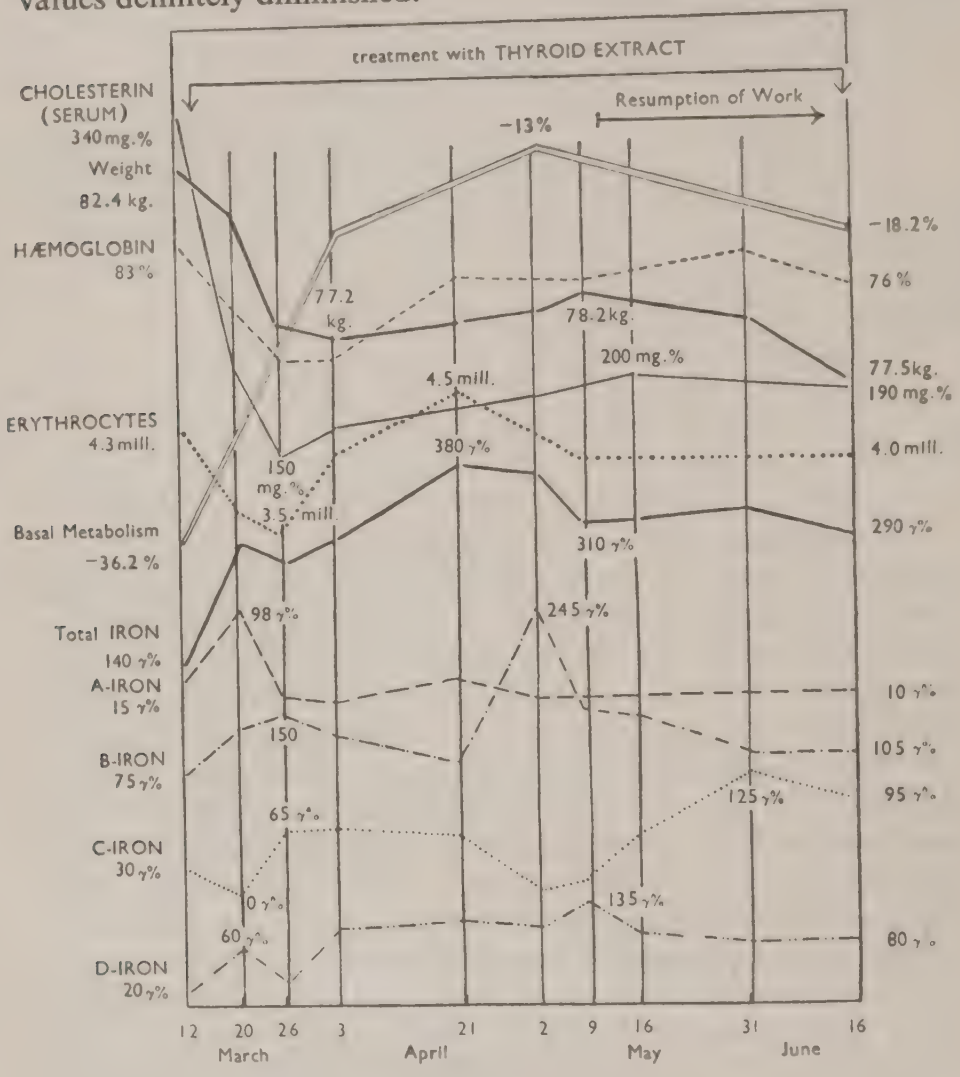

DIAGRAM 11
Typical case of myxoedema in a man of 48.

From the above facts we can draw the following conclusions: A stimulation of the general metabolism by thyroid extract is followed by a rapid increase of the separable iron which, as some authors have already emphasised, is accompanied by a certain degree of haemolysis (increased bilirubinaemia and slight anaemia). Here we see a reaction similar to that found while an organism is becoming adapted to high altitudes. In order to meet the requirements of a cell upon whose activities an excessive demand is being made, the body mobilises its reserves, and, in the absence of these,

it creates a more or less definite haemolysis, whereby it is enabled to utilise the iron of the haemoglobin for other purposes. This would explain the slight rise of bilirubinaemia sometimes observed at the beginning of thyroid treatment in cases of hypothyroidism.

The marked increase of the total iron during the third phase was doubtless caused by improved intestinal absorption of iron. The favourable effect of thyroid treatment on the gastric hypohydria or achlorhydria in hypothyroidism is well known. This achlorhydria is to be partly attributed to the anaemia and lack of iron in myxoedema. We will revert to this subject later. The favourable effect of the thyroid treatment on the gastric secretion is conspicuously indicated in the total iron, which showed a distinct rise, while the separable iron was stabilised at lower values. The iron mobilised during the second phase of intensive treatment was in part utilised at the periphery, where it developed its activity as a biocatalyst in order to support the activated cell oxidation, and in part it served to neutralise the incipient anaemia in the bone-marrow.

Finally, during the fourth phase, after the condition of the organism had improved sufficiently to enable the patient to resume his daily work (heavy physical exertions of a manual worker), the fractions of the separable iron, as well as the total iron and the values of the haemoglobin greatly declined. In an individual who, during repose, can be partly treated by thyroid extract, muscular exertion has an unfavourable effect on the iron metabolism, and indirectly also on the basal metabolism. The iron reserves are still too insufficient to be able to assist in accelerated muscular activity; consequently there is a distinct reduction of all the iron fractions, accompanied by anaemia and a renewed decline of the basal metabolism, even though the patient still continues to be under the influence of the same doses of thyroid extract.

In hypothyroidism, therefore, we are generally concerned with a reduced iron content of the serum and tissues. This may proceed on the one hand from faulty iron absorption due to lack of hydrochloric acid in the gastric juice, and on the other hand from a reduced need of help in respiration on the part of the tissues. The thyroid treatment affects the mobilisation of iron, which may involve a compensatory haemolysis; but it in its turn is followed by a new reduction of the serum iron value due to consumption of this iron, either as a biocatalyst or as a substance of blood synthesis.

According to the phases during which we analysed the iron metabolism in hypothyroidism in the course of thyroid treatment, we observed either an increase or a reduction of the circulating iron. Usually before treatment there was a reduction of the serum iron

content, after which, upon the addition of thyroid, a certain amount of iron was mobilised; next, as soon as the treatment produced the clinical symptoms of an activation of tissue metabolism or of erythropoiesis, renewed diminution of the serum iron content could be noted.

The problem of the regulatory relations existing between certain forms of anaemia and of hypothyroidism has been associated by *Vannotti* with iron metabolism in connection with the disturbances of thyroid activity. The influence of the thyroid on the functioning of the bone-marrow and upon erythropoiesis in general is not yet fully clarified, although clinical and experimental experiences have frequently furnished various instances indicative of a relationship existing between these two systems which appears to be pre-eminently hormonal in character. *Heilmeyer* and his co-workers have shown that, if the thyroid of animals is removed, the number of red blood cells falls and the haemolysis gradually declines. Treatment with thyroxine prevents this from occurring. In thyroidectomised animals the erythropoietic reaction to various stimuli is considerably arrested. In a publication dealing with the effect of the thyroid on the blood system, *Boccuzzi* and *Paolino* stress the fact that the numerous clinical observations bearing on this subject present no definite conclusions. Indeed, while in many cases there is an increase of red cells, in exophthalmic goitre (31% of cases, according to *Kleiner* and *Frenreisz*) several authors describe more or less clearly defined anaemia in hyperthyroidism (15% of the cases of exophthalmic goitre, according to *Kleiner* and *Frenreisz*).

In myxoedema most authors note the existence of anaemia. In such cases it usually assumes the form of secondary anaemia which is often very severe, especially in juvenile myxoedema. Hyperchromic anaemia, on the other hand, is less frequent (*Holboll, Meulengracht, Sharpe*). In certain cases the development of typical pernicious anaemia has even been noted in myxoedema (*Mackenzie, Griffin* and *Bowler, Boros* and *Croniser, Lesser* and *Andersen, Holboll, Andrus, Cowles* and *Wintrobe*, etc.).

In one case described by *Vannotti*, pernicious anaemia developed rather quickly at the age of 20; not until a year and a half later did hypothyroidism make its appearance. Myxoedema and pernicious anaemia remained even after a long period of treatment, but systematic therapy with thyroid extract permitted the patient to abandon the hitherto indispensable liver treatment. A continuous treatment of thyroid extract for a month and a half was followed by a slight exacerbation of the anaemia. In a second case, that of a patient of 25, the same author observed the simultaneous appearance of hypothyroidism and pernicious

anaemia. This same syndrome was recently seen again in a young person.

The problem here confronting us was whether the myxoedema and pernicious anaemia represented two conditions which chanced to occur at the same time or whether they were in any way aetiologically connected, in the sense that one might have been a complication of the other. Some authors, as a result of observations (although not numerous) tend to consider the idea of mere chance as not very probable, especially as the two syndromes are comparatively rare. We personally reject the idea of chance for the reason that treatment with thyroid extract frequently has a favourable effect on the course of the pernicious anaemia.

The hypothesis that there might be some possible connection between the thyroid hypofunctioning and the pernicious anaemia strikes us as much more plausible. It may be that constitutional factors play a certain part in the simultaneous development of hypothyroidism and pernicious anaemia. As certain authors (*Green, Sharpe*) have already observed, myxoedema and pernicious anaemia often show the same symptoms: the colour and type of skin, sometimes atrophy of the tongue, a reduction in the number of reticulocytes, particularly in old cases of myxoedema. Finally, achlorhydria, which is a constant phenomenon in pernicious anaemia, often occurs in severe hypothyroidism. Anaemia is a familiar symptom in hypofunctioning of the thyroid, although it usually takes the form of secondary anaemia, for hyperchromic anaemia is unusual in myxoedema. As regards experimental hypothyroidism, *Sharpe* found that thyroidectomy in the rat provoked a form of anaemia characterised by an increase in the volume of the erythrocytes. This anaemia reacted neither to iron nor to liver therapy, but vanished in response to injections of thyroid extract.

The view that hypofunctioning of the thyroid constitutes the aetiological cause of pernicious anaemia appears to us to be too far-fetched. Myxoedema certainly will sometimes develop on a basis of a pernicious anaemia which has been fully developed for several months or even a year, and cases of pernicious anaemia have been observed which have been associated with hyperfunctioning of the thyroid in exophthalmic goitre (*Boccuzzi* and *Paolino, Meulengracht* and others).

According to *Bomford*, pernicious anaemia is the result of a simultaneous insufficiency of both iron metabolism and hepatic functioning, which are to be considered as indirectly caused by the presence of the myxoedema. He holds that the achlorhydria of the myxoedema arrests the intestinal absorption of the iron, and that this in turn leads to the development of a secondary form of anaemia or to severe iron-deficiency anaemia.

The pernicious anaemia would thus be the result of the co-operation of faulty iron absorption with a hepatic injury, and, in a last analysis, of defective cell respiration caused by the hypofunctioning of the thyroid. This functional co-operation of the extracts of thyroid and liver, that is of the thyroid and liver themselves, was demonstrated experimentally by *Mansfeld*, who emphasised the important role played by the thyroid in activating the anti-pernicious principle of the liver. It still remains to be determined whether this collaboration operates directly or only indirectly. In this connection we consider it opportune to recall our remarks on iron metabolism in hypothyroidism (in the last-mentioned patient). In our opinion iron represents, so to speak, the connecting link between two biological processes of great significance. The first process represents the activity of iron in its capacity as the oxygen-carrier in the blood, which activity is associated with the function of the bone-marrow and is thus dependent upon the hepatic function; the second embodies the significance of iron in cell respiration, which is indirectly influenced by the thyroid. Support for this view is found in the recent descriptions of the relationship existing between the thyroid and lactoflavin (*Wahl*).

On the one hand, iron deficiency may reduce the activity of the bone-marrow and provoke an anaemia; on the other hand, it may impede certain processes of tissue respiration. The clinical picture of peripheral anaemia of the circulation would therefore be the manifestation of an anaemia of the tissues, that is, a diminution of the "haem" associated with the presence of iron and which are necessary to retain the circulating oxygen and to furnish it with the cellular substrate. Fortunately the human body is able to prevent cellular respiration insufficiency. Thus it comes that certain forms of anaemia, while showing a great deficiency of circulating iron, nevertheless have considerable iron reserves in the tissues. But, if the iron deficiency is very great and the iron reserves of the tissues begin to vanish, some evidence of insufficient respiratory exchanges would have to be apparent in the cell. The body reacts to this grave situation by mobilising other neutralising catalysts or regulatory mechanisms. Thus it may happen that in such forms of anaemia a renewal of thyroid activity in the form of hyperthyroidism is sometimes observed.

Pernicious anaemia, on the other hand, is the manifestation of a disturbed erythropoiesis in which there is an accumulation of insufficiently utilised iron in the circulating blood and tissues (which as a result become over-supplied with iron). In this form of anaemia it would not surprise us to note in the tissues occasionally a definite increase of the catalytic activity of the iron, since processes of respiration can be stimulated by the accumulation of

biologically active iron. This view would account for the rise of the basal metabolism in pernicious anaemia (according to *Suzmann* and others). Nor is it even excluded that in certain special cases, based on a particular type of constitution, the presence of considerable amounts of active iron in the tissues might serve to diminish, in a compensatory manner, the thyroid activity which is exerting a stimulating effect on the cell respiration. In such cases, although of rare occurrence, we should witness the development of hypothyroidism as a secondary condition of the pernicious anaemia.

The hypofunctioning of the thyroid would in its turn involve either directly or indirectly a suspension of the functions of the stomach, liver and bone-marrow, as a result of which, of course, the general reaction of the organism would deteriorate in the pernicious anaemia. In such a case the thyroid extract which stimulates the iron metabolism, the activity of the bone-marrow, and the gastric secretion, will often alone suffice to re-establish a certain physiological equilibrium and to arrest the progress of the pernicious anaemia. Thus, in our first case, the non-haemoglobin serum iron which increased as a result of thyroxin treatment rapidly returned to normal values. The liver extracts, on the contrary, were unable to act on the thyroid. In these cases, therefore, the treatment should consist of a combined thyroid and hepatic therapy. The method of co-operation of these two extracts is explained in the observation of *Mansfeld*, who found that if rabbits previously thyroidectomised were rendered anaemic with saponin the hepatic extracts had no effect.

The observations of *Mansfeld* deserve to be explained in more detail. A certain lack of oxygen led to a direct increase of thyroid activity (without the intervention of the pituitary body) and at the same time increased erythrocyte formation. Now does there exist any relation between these two reactions? Whatever the answer, the effect of the high altitude on blood formation is seen only in normal animals; it is absent in thyroidectomised animals. Only in those possessing their thyroid does regeneration proceed rapidly in cases of haemorrhagic anaemia or in phenylhydrazine- or saponin-anaemia. In animals without a thyroid the liver extract does not operate, whilst extracts of the gastric mucous membrane retain their complete potency. Hence the anti-pernicious principle of the liver must be activated by a principle of the thyroid before it can exert its effect on the bone-marrow. This principle of the thyroid, in addition to thyroxine, is present, according to *Mansfeld*, in the acid-free glandular extract. These conclusions, which are of considerable practical importance, still lack confirmation by other observers; but even now they show us the importance of the

relationships existing between the thyroid (and hence general metabolism) and the liver as the regulator of erythropoiesis, which relationships are closely connected with iron metabolism.

We now come to a discussion of our iron investigations in hyperthyroidism, and begin by quoting some examples:

Mrs. M. J., aged 36. Basal metabolism, +79.8, +72.9%.
Barkan iron, 32 γ%. Heilmeyer iron, 110 γ%.
After a few weeks' treatment there were definite signs of subjective and objective improvement.
Increased weight, 5 kg. Reduction of basal metabolism, +65.3, +57.9%.
Barkan iron, 56 γ%. Heilmeyer iron, 157 γ%.

Mrs. D. M., aged 37. *Typical exophthalmic goitre.*
Barkan iron, 10 γ%. Heilmeyer iron, 46 γ%.

Mrs. N. C., aged 30.
Before treatment: Basal metabolism, +56.9%.
Barkan iron, 0 γ%. Heilmeyer iron, 30 γ%.
After treatment resulting in subjective and objective improvement:
Barkan iron, 20 γ%. Heilmeyer iron, 96 γ%.

Mr. M. F., aged 34.
Basal metabolism, +19.8%. Hyperthyroidism.
Barkan iron, 5 γ%. Heilmeyer iron, 72 γ%.

Mrs. B. E., aged 29. *Incipient exophthalmic goitre.*
Basal metabolism, +35%. A-iron, 5 γ%. B-iron, 245 γ%. C-iron, 65 γ%. D-iron, 40 γ%. Total iron, 355 γ%.

Recently we observed in experimental hyperthyroidism of the rabbit that injected radio-active iron is fixed in the bone-marrow in extremely high concentration, whilst in the reserve organs (liver and spleen) the iron level is particularly low. The iron content of the muscles is normal. Therefore the circulating iron is low. This enormous mobilisation of iron in the bone-marrow explains the important variations of iron metabolism in hypo- and hyperthyroidism.

In hyperthyroidism, just as in hypothyroidism, no exact conclusions can be drawn from one single iron determination. These depend alone upon the intensity and the stage of the illness during which the iron determination was made. In the last-reported case the condition was one of incipient exophthalmic goitre (clinical appearance of the disease three weeks previously); here we see a distinct increase (mobilisation) of the B-iron, whilst in the other cases, corresponding to a protracted increased consumption of iron in the periphery (rather to be considered as chronic cases) we find a slight reduction of the non-haemoglobin iron level in the serum. The slight increase of the serum iron content accompanying the decrease of the basal metabolism following upon the treatment for exophthalmic goitre seems to be an expression of a partial diminution of the body's need of tissue iron.

The above-mentioned relationships between thyroid and erythropoiesis are surmised to exist not only in hypothyroidism, but also in hyperthyroidism, as is shown by the following case which we wish to discuss here in detail.

The patient was a girl of about 18 who since childhood had been suffering from slight secondary anaemia. Due to a long period of overwork, the anaemia became exacerbated a year previously (Hb. = 50%). A rest cure brought no improvement, but during that time a condition of hyperthyroidism became gradually manifest, accompanied by tachycardia, nervous disturbances, frequent perspiration and trembling. The patient attended the medical Polyclinic of the University of Lausanne, where we were able to determine the typical symptoms of exophthalmic goitre (pulsating goitre, pronounced exophthalmus, tachycardia, tremor, etc.), although the general condition was still good. But the blood analysis indicated pronounced secondary anaemia. Hb., 35%. Erythrocytes, 3,800,000. Bilirubinaemia, 0.3 mg.%

To our surprise three successive checks of the basal metabolism[1] showed values of between +2.5 and +8.8%, viz. normal values, which conflicted in a striking manner with the clinical picture of our patient. The blood analyses showed a decided reduction of the serum iron. We treated our patient with considerable quantities of iron per os plus hydrochloric acid and pepsin, and with daily intravenous injections of Ce Ferro. The anaemia did not react in a perceptible manner to this intensive iron therapy; but, on the other hand, the exophtbalmic symptoms augmented, the patient lost weight and the values of the basal metabolism rose and, as soon as the serum iron values increased, began to assume the clinical picture of exophthalmic goitre. Later we observed a further improvement of the anaemia, but the severe symptoms of Graves' disease compelled us to interrupt the iron treatment. From that time onwards the clinical picture of the exophthalmic goitre showed a slow but progressive improvement. (See table on page 218.)

This interesting case, which we were able to follow up systematically, offers a clinical contribution to the problem of the catalytic activity of the iron in the chemical processes, and at the same time reveals the dual significance of iron activity in connection with erythropoiesis and the cellular chemical processes, respectively. In this case we see hyperthyroidism reinforced by hypochromic iron-deficiency anaemia, with very low serum iron values. During the period of severe iron deficiency we see the complete clinical picture of exophthalmic goitre, but this is accompanied by no increase of the basal metabolism, although it was estimated three times. Thus the findings of the metabolic examination contrast violently with the clinical symptoms. As soon as we administered iron in large doses, leading to the temporary partial concealment of the iron deficiency, we noted, not an improvement of the anaemia, but a distinct rise of the basal metabolism. This observation shows us that a lack of iron as a catalyst of the oxidation and as a regulator of the cellular chemical processes may prevent the increase of the basal metabolism and the emaciation typical of exophthalmic goitre. The large doses of iron administered were utilised chiefly as a compensation for the lack of tissue iron and at the expense of

[1]The analyses of the basal metabolism of this patient were carried out in the laboratory of the Medical Clinic of the University of Lausanne. We take this opportunity of expressing our sincere thanks to *Prof. L. Michaud* for his friendly co-operation.

haemoglobin formation. The anaemia was not affected but the basal metabolism rose and the patient became thinner. In this case, therefore, the main effect of the administration of iron was to stimulate the cellular metabolic functions.

Here are the results of the analyses of the observation on page 216:

	Weight Kg.	Basal metab'm	Hb. %	Erythrocytes Mill.	Bilirubinaemia mg.%	Barkan iron γ%	Heil'r iron γ%
Before treatment	58.6	+ 8.8	35	3.8	0.30	0	10
After 12 days of ferrum reductum	57.0	+ 2.5 +20.5 + 8.4	34	3.4	0.48	0	52
After 5 days' interruption of treatment per os	57.0		38	4.0	0.49	0	17
After 14 days of treatment with 6 mg. of iron daily intravenously	56.0		40	4.3	0.34	10	91
After 5 days' interruption of iron treatment		+19.0 + 9.6	38	4.0	0.38	0	16
After 8 days of treatment with 10 mg. of iron intravenously each day	55.2	+34.2 +24.4	42	4.4	0.41	20	80
After 10 days without treatment	54.3		38	4.0	0.31	10	84
30 days later (without iron treatment)	57.0	+15.2 + 5.3	37	4.0	0.30	0	22

This case also shows us that for conditions of diminished iron content in the tissues (tissue anaemia) the administration of iron serves chiefly to conceal the deficiency of iron possessing biocatalytic activity; in such cases the organism appears to take the needs of erythropoiesis into only secondary consideration.

These various facts permit us to assume that the systematic examination of iron metabolism may be of great clinical importance not only in determining the various processes involved in erythropoiesis and in iron absorption and excretion in both normal and diseased organisms, but also in studying the regulation of the peripheral oxidation and the chemical processes of the tissues in general.

VII. General Conclusions

The object of this work has been to offer a critical analysis of normal and pathological iron metabolism on the basis of numerous determinations of non-haemoglobin iron. We wish to draw some general conclusions. At the same time, the data obtained from the various chapters will be arranged in such a form as to present a comprehensive picture.

The study of iron metabolism in human beings presents a many-sided problem and one that is associated with great difficulties. These are in the first place technical in nature, since the quantitative determination of iron is a complicated process and the methods available are still often inadequate. It is sometimes difficult to estimate the significance of the various results obtained by iron determination, for we are concerned with different iron fractions of unknown origin which do not always represent clearly defined units, considered from a chemical or biological standpoint, but are frequently the product of a particular method of extraction.

Hence it would be erroneous to attribute *a priori* a special biological individuality to each isolated iron fraction and from this to deduce conclusions based on an iron fraction thus characterised. Not until the entire problem has been envisaged in detail and until the results of the iron determination obtained by one method are compared with the clinical or experimental findings of processes with a well-understood mechanism, will clarification be obtained. This difficult and multifarious problem is variously and intricately interrelated with a number of problems of general biology, physiology and human pathology, for which reason it is of special clinical and practical significance.

In the circulating blood there are two great iron fractions: (*a*) the iron of the blood pigment formed by a porphyrin ring containing in its centre an atom of iron, i.e. the haemoglobin iron; (*b*) the iron which circulates, together with the haemoglobin iron, in the plasma and blood corpuscles, i.e. the non-haemoglobin iron.

The haemoglobin iron is of no special interest in our investigations; its metabolism is that of the haemoglobin molecule, which was discovered long ago with the help of quantitative determinations of the blood pigment in the domains both of physiology and of human pathology. Non-haemoglobin iron, on the other hand, was long disregarded and not until the end of the nineteenth century was iron discovered in the blood which was not associated with haemoglobin.

From a practical point of view non-haemoglobin iron in the body can be divided into two groups: cellular iron which serves as

material for tissue synthesis and is found in the blood cells as well as in all other living cells; and the circulating iron which travels in the blood stream and which can also be designated as transport iron to differentiate it from the circulating haemoglobin iron. This iron, which is found chiefly in the plasma and hence also in the serum, is of great clinical interest, as it often reflects the complex metabolism of iron in the organism.

Among the fractions of circulating iron on which we have concentrated our particular attention are the non-haemoglobin irons, and more especially the serum iron, which even prior to and simultaneously with our own investigations (*Vannotti*, 1937) constituted the subject of numerous publications. Very extensive and important contributions in this field of effort have been published by *Barkan* (1927), *Starkenstein* (1928), *Heilmeyer* and *Plötner* (1937) and *Vahlquist* (1941).

Iron does not exist in a free state (ionised iron) in the serum, but always in the form of chemical complexes. By the addition of HCl it can be partially liberated from its organic connections. This iron we designate as " easily-split-off iron ". The quantity of iron liberated from these complexes will vary in accordance with whether a weak acid (such as trichlor-acetic acid) or a strong acid (hydrochloric acid) is used, and depends on the concentration of the acid. This circumstance explains the various values of the fractions described by *Barkan, Heilmeyer, Starkenstein* and ourselves. According to the acid used, and its concentration, more or less of the iron bound in complexes is liberated. But, in addition to this iron that can be separated by a weak acid, there also exists in the serum another iron fraction which cannot immediately be separated by the acid. This, therefore, must be iron that is so closely bound as a complex that it cannot be freed by the 6N HCl (from amino acid, sugars, oxyacids, phosphates) or cannot be coloured with thiocyanate. This non-separable iron remains in solution in HCl in the form of a complex from which it can be determined by incineration. Another part remains bound to the protein bodies of the serum or to other substances (protein-carriers) either because it belongs to the protein molecule or because it is adsorbed by the protein. This is the " iron of the protein precipitate " (iron that can be precipitated with the protein). In order to be able more accurately to follow the variations of the serum iron we undertook to determine the four following fractions:

A-iron (Iron fraction A): Iron of the easily split-off complexes = lightly bound iron. A weak acid (trichlor-acetic acid) suffices to liberate it.

B-iron: Iron of the not easily split-off complexes = firmly bound iron. This can only be freed by concentrated HCl.

C-iron: Iron of the complexes which is soluble in acid and non-

separable or not producing a colour with the thyiocyanate method. It can only be shown up after the HCl extract has been incinerated.

D-iron: Iron of the protein precipitate (which can be precipitated with the protein).

These iron fractions together represent the total serum iron. The A-iron is approximately the same as *Barkan's* iron, the B-iron is very similar to *Heilmeyer's* iron. These four fractions do not constitute sharply defined biological and chemical units, but rather represent various portions of iron characterised by different physico-chemical properties which, in the course of iron metabolism, appear regularly in the organism and hence possess a certain biological importance.

Normal Iron Metabolism

With the help of these iron fractions we next proceeded to a study of physiological iron metabolism, starting with the problem of iron intake and taking as our point of departure the important investigations of *Starkenstein* and his school.

In addition to the considerable iron reserves with which the organism is endowed at birth and which accumulate in the body, principally during the last few months of intra-uterine life, the organism derives its iron from nutrition (at first in the mother's milk) through absorption from the digestive tract.

Iron absorption and excretion. Only part of the dietary iron is absorped from the digestive tract. It is principally the ferrous salts, i.e. the reduced iron, which penetrate the intestinal wall. This reduction is effected in the stomach with the help of the gastric juice (and above all of the hydrochloric acid) which, in the opinion of *Lederer*, contains an enzyme having power to separate iron from its organic compounds and complexes. Iron absorption occurs in the duodenum. Once the intestinal wall has been traversed the ionised reduced iron attaches itself to complexes and enters the circulation. The rate of the absorption is not proportional to the amount of nutrient iron present in the duodenum, but depends greatly upon the body's need of iron. Hence there exists a very important system of control whereby the iron metabolism is regulated, aiming, on the one hand, to prevent too great an accumulation of iron in the organism, and on the other hand, to increase the iron absorption to meet any given needs. This graduation of iron intake is doubly important in view of the fact that iron excretion is not the prime factor in regulation, as might have been expected. The regulation of iron absorption by the intestine should be done through the medium of ferritin, a specific iron-protein complex (*Granick* and his collaborators). The

organs of excretion are the intestine (especially the colon) and the kidney which, in certain cases, can avoid a too great increase of non-haemoglobin iron by a rapid elimination. The bile also serves to excrete iron, although in this case the iron does not necessarily leave the body. As we shall see later, it can be absorbed again and led back into the circulation.

Conversion of the absorbed iron. The iron absorbed from the intestine into the blood circulation is rapidly joined to the plasma proteins. To-day we know that the β globulin (*Cohn*) is the specific carrier of iron. Adsorption and chemical combination to form complexes are necessary to incorporate iron in the proteins. Therefore, iron transport may be related to the percentage of proteins in the plasma and to their quality. In this case, the non-haemoglobin iron varies not only according to the metabolic needs of the organism, but also according to the compoistion of the plasma proteins and the physico-chemical facts which regulate their function (iso-electric point) and which are expressed clinically by the three following factors: percentage of plasma proteins, ratio between albumin and globulin, sedimentation rate of the blood cells.

Moreover, the non-haemoglobin iron depends on the intensity of the iron absorption, on the requirements of the organism for the formation of haemoglobin; it depends also, for the utilisation of iron in the tissues, on the mobilisation of iron stores, on the intensity of destruction of the blood cells, etc.

The most important organ for iron metabolism is the liver, which takes up a great quantity of circulating iron and transforms it; it can act as storage organ or as excretory organ through the bile. The spleen accordingly plays in iron metabolism only the subordinate role of an organ of storage for the final product of conversion of this metal.

By utilising radio-active iron, *Whipple* was recently able to demonstrate that the iron which is absorbed by the intestine is conveyed in the plasma to the organs of consumption. The liver and bone-marrow promptly retain the major part of the absorbed iron; at the same time a rapid passage of the iron from the plasma to the erythrocytes can be noted. The latter, therefore, also serve to transport the iron.

Biological significance of iron. As an indispensable constituent of the cell, iron co-operates in the formation and growth of the organism. It also plays a part in the construction of the haemoglobin molecule, which it converts into a substance necessary for haematopoiesis.

Finally, iron is closely associated with respiration and with the general chemical processes of the cell, both as an oxygen-carrier

in the haemoglobin and as a catalyst in numerous cellular chemical processes (cell oxidation and chemical tissue exchanges).

As an element of synthesis iron is present in all the tissues. The newly-born possess numerous well-stocked places of iron storage, particularly in the liver. These supplies become gradually reduced as the body develops.

Haemoglobin synthesis proceeds from the chromogen by the introduction of iron into the porphyrin ring. This synthesis takes place in the erythroblast, where it is probably carried out by the iron-containing cytoplasm (*Vannotti*). Thus the nucleus of the erythroblast would be the agent that conveys the iron atom to the porphyrin ring. This concept would explain the karyolysis occurring in haemoglobin synthesis in the erythroblast, as well as the appearance of haemoglobin-containing but nucleus-free erythrocytes in the blood stream.

The mother-cells of the erythroblasts in the bone-marrow contain no haemoglobin. Indeed, during the first few months of embryonic life porphyrins are found in the bone-marrow in place of haemoglobin. During this period, therefore, the bone-marrow is not capable of carrying out haemoglobin synthesis; blood pigment formation does not proceed beyond the formation of an iron-free product representing a porphyrin. This retrogression to the embryonic conditions of erythropoiesis is found again in pernicious anaemia, in which state the reappearance of the megalocytic form of the red blood cells (characteristic of early embryonic life) is accompanied by the appearance of porphyrin.

Finally, iron plays a very important part in the regulation of the chemical processes, especially of tissue respiration. The mechanism of cell oxidation which has been studied in detail by *Warburg* and his school, as well as by *Wieland, Keilin* and others, is controlled by a number of cell enzymes and pigments, among which iron plays a prominent role. Among the iron-containing cell-constituents of the cell partaking in the cellular chemical processes should be mentioned *Warburg's* respiratory red enzyme, peroxydase, catalase, cytochrome, etc. The biocatalytic faculty of iron relates in particular to the power possessed by certain of its compounds to alter the valency, in accordance with whether oxygen is taken up or given off. By losing oxygen trivalent iron becomes converted into divalent iron, and this divalent iron, under the influence of a medium of oxidation, can in turn be re-converted into a trivalent compound, without greatly altering the medium in which the catalysis occurs.

By this means iron assists and controls respiration and the other chemical processes of the cell. It is thus endowed with a function indispensable for tissue life, which is as important

as its participation in cell formation and the synthesis of blood pigment.

Among these important cellular constituents particular mention should be made of cytochrome which, biochemically considered, shows certain analogies with haemoglobin. This pigment is actually formed by a porphyrin with which iron associates itself. This therefore represents a type of cell-haemoglobin found in varying concentrations in the tissues. In addition to cytochrome, in the striated muscle there is another haemoglobin which is still more closely related to the blood haemoglobin. This is myoglobin.

Every cell contains small quantities of iron in its cytoplasm. It is assumed that the object of this iron is to bind the corresponding chromogen which is characterised by the porphyrin ring. Thus each cell might form a haemin, as a result of which in the erythroblasts of the bone-marrow there would take place the synthesis of haemoglobin; in the other cells, that of cellular haemin, or cytochrome, and in the muscular tissue the formation also of myoglobin. All three substances, which show a similar chemical constitution (porphyrin + iron), owe their biological significance as oxygen-carriers to the presence of iron in their molecule.

To-day we are familiar with all the details of normal and pathological haemoglobin metabolism as well as with the pathological pictures which are produced by reduction or increase of this pigment. But we are ill-informed regarding possible disturbances of these two cellular haemins (cytochrome and myoglobin) which are no less important than haemoglobin. Indeed, it is by no means excluded that in addition to an *anaemia of circulation* (haemoglobin-deficiency) there also exists a *tissue anaemia* (cytochrome and myoglobin deficiency) caused by lack of iron in the tissues, a condition which would involve a certain insufficiency of cell respiration.

We deem it a matter of some interest to mention in this connection the hypothesis of *Amano* who, having determined the presence of large quantities of cytochrome in the eosinophilic leucocytes, assumed that these cells might be conceived as sites for the transportation or deposit of this pigment in the circulating blood.

Pathological Iron Metabolism

We have offered a general picture of the iron problem, as seen to-day by the eyes of the observer and clinician. We now propose to summarise our observations from the field of pathology. The following are the pathological conditions on which our attention has mainly centred.

Haemolysis. Haemolysis is accompanied by a definite increase

of A- and B-iron, i.e. of the separable iron. This is revealed first of all in the erythrocytes, next, as a result of diffusion, in the plasma and serum. At the same time a certain reduction of the C-iron is observed, that is, the iron of the non-separable complexes, and an increase of the iron of the protein precipitate, corresponding to the passage of the haemoglobin into the serum. The augmented destruction of the red blood corpuscles thus frees the newly absorbed iron which, according to *Whipple,* promptly attaches itself to the erythrocytes and probably also to other iron fractions which are bound up in a varying degree with complexes and are brought into circulation by the red blood cells. The reduction of the C-iron is probably due to the fact that further transformation of iron by the overlooked reticulum is inadequate, whilst the increase in D-iron is due to the increase of the protein-containing products of destroyed erythrocytes and to the passage of haemoglobin into the plasma.

In chronic haemolysis these variations are less perceptible, although usually there can be noted a tendency on the part of the plasma to become enriched with iron of the separable complexes.

Hence the degree and intensity of the haemolysis determine the serum iron, both quantitatively and qualitatively. In acute haemolysis the increase of the A and B fractions is only very transient and is often followed by a marked decline; sometimes this already sets in after six hours and is probably caused by a rapid consumption of iron for the formation of new blood.

The anaemias. In discussing our investigations in the domain of iron metabolism in the various forms of anaemia we limited ourselves to a somewhat imperfect sub-division of the anaemias. This, however, possessed the advantage of emphasising the significance of iron metabolism in the aetiology of these blood diseases.

The designation " Iron-deficiency anaemia " originated with *Heilmeyer*, who, in a number of important publications, furnished evidence of a considerable reduction of the serum iron level in various forms of anaemia. Among those showing a reduced iron level are to be included the post-haemorrhagic anaemias, the anaemias resulting from insufficient dietary iron intake, anaemias caused by iron being utilised for purposes other than haemoglobin formation (post-infectious anaemias), and finally, idiopathic hypochromic anaemia and the iron-deficiency anaemias, produced by a number of jointly operating causes.

The acute post-haemorrhagic anaemia shows a corresponding reduction of all the iron fractions. This is due to loss of blood and to the ensuing dilution through water mobilisation, whereby the body seeks in part to compensate for the deficiency in the total

volume of circulating blood. Careful observation will show, however, that the relationship between the various fractions is soon destroyed, since the iron value of the separable complexes diminishes more than does that of the other fractions. This great reduction of irons A and B can be explained in the same way as in the case of haemolysis, and it can be observed more easily and for a longer period in those forms of anaemia that are caused by chronic haemorrhage. This diminution is sometimes so considerable that it can no longer be accounted for by the loss of blood and by the mobilisation of iron for the work of forming new blood in the bone-marrow; for blood loss and regeneration are often far from being in proportion to what may be a severe degree of anaemia. Thus everything contributes to the assumption that the " easily split-off ", biologically active iron is not only mobilised by the bone-marrow for the work of erythropoiesis, but also by the tissues, which have difficulty in maintaining normal respiration and hence require catalytic iron in order to support the imperilled gas exchanges. In this form of anaemia there is often very extensive mobilisation of the depository iron. The iron supply of the spleen is often reduced to a very conspicuous degree, the total serum iron diminishes considerably, whilst the tissues for a long time maintain intact their iron endowed with catalytic powers. Thus a possible reduction of the iron content of the tissues is only effected long after the serum iron has been reduced.

These forms of anaemia react well to iron therapy. Peroral administration is accompanied by marked absorption and retention of the iron by the organism.

Anaemia caused by insufficient intake of iron usually takes the form of an anaemia of nutrition, a condition sometimes found among the poorer classes, especially in persons who do not follow a rational system of diet. But in addition to this deficiency in dietary iron there are also forms of anaemia showing a reduced serum iron content, occasioned by a defect in the iron absorption through the intestine. This is usually what occurs in chronic gastroenteritis, achlorhydric gastritis, and in certain instances of gastroenterostomy and gastric resection.

Observations on experimental anaemia caused by deficiency of dietary iron show that the iron deposits of the spleen undergo practically no change at all, and that it is chiefly the liver which mobilises its reserves. On the other hand, the iron of the muscles and myocardium, that is of the organs which are called upon to carry out the greatest feats of energy, remains unchanged for a long time, and even in the course of protracted iron deficiency does not show much reduction.

Another form of anaemia with reduced serum iron content is

caused by the utilisation of the iron for purposes other than haemoglobin formation. These forms are pregnancy anaemia, in which the mother supplies the foetus, chiefly during the last months of pregnancy, with considerable quantities of iron; the anaemia of growth; certain anaemias of childhood and puberty; anaemia arising in connection with the development of malignant tumours; and the anaemias of acute and particularly chronic infectious diseases. In the last-named form, we observe a rapid passage of iron into the storage organs and an inhibition of haemoglobin synthesis with production of protoporphyrin. These facts are probably due to a disturbance in the formation of globin (*Wintrobe*) and to the fact that the quantitative and qualitative alterations of the plasma proteins prevent normal transport of the iron to the bone-marrow.

Idiopathic hypochromic anaemia or achylic chlorotic anaemia constitutes a disease *per se*, with a clearly defined clinical picture. It develops mainly in women in the forties and is characterised by anisocytosis and microcytosis, leukopenia and gastric achylia. The phenomena which commonly accompany this form of anaemia are digestive disturbances, atrophy of the tongue, rhagades around the corners of the mouth, very brittle finger nails, paresthesiae, menstrual disturbances and splenomegaly.

The aetiology is multiple. In addition to constitutional and hereditary factors the anamnesis often shows an augmented loss of blood and iron (pregnancies, menorrhagia), disturbances in iron absorption caused by lack of hydrochloric acid, probably sometimes also by the absence of a factor needed to liberate the dietary iron (*Lederer*), and diminished iron absorption due to excessively rapid passage through the small intestine of nutrient substances (*Thiele*). This form of anaemia shows a very low content of A- and B-iron, that is, of the separable iron. As a rule there is a great difference between the low values of the separable iron and the often normal C and D fractions; hence the total iron usually registers only a slightly reduced value. In this anaemia, therefore, the iron deficiency would be only apparent. It would seem to be considerable if only the A- and B-iron, or the *Heilmeyer* and *Barkan* iron, were determined; but it is often only slight when a determination is made of the total iron. In such cases, therefore, it must be assumed that there exists an abnormal distribution among the various iron fractions, and this applies particularly to the separable iron; for a lack of this iron, due to its biological importance as a determining factor in the chemical processes of the cell and of normal blood formation, is the cause of some of the symptoms of this anaemia. In this form of anaemia there can often be noted a curious inertia of the bone-marrow in relation to iron treatment.

Sometimes the anaemia will improve for several weeks or even months after both peroral and parenteral iron treatment; frequently after peroral administration there is excellent iron absorption, even if the gastric juice lacks hydrochloric acid. It is highly probable that this chronic form of anaemia is the manifestation of a systematic impoverishment of the organism in separable iron, which may even affect the tissues. This would thus be an instance of " tissue anaemia ", and this fact would serve to explain quite a number of clinical symptoms, such as the slight fatigue, the adynamia of these patients and certain lesions of the skin, mucous membrane and nails—general and localised symptoms which often quickly disappear in the course of the iron therapy before the anaemia can be permanently cured. Thus it appears that the iron is utilised to meet the needs of cellular functioning before it is used by the bone-marrow, and only in the third instance would it be called upon to supplement the deficient reserves. Indeed one is often astonished to note that anaemias which react well to iron and which one is tempted to consider cured will rapidly relapse if the treatment is interrupted too soon.

We should like at this point to mention the appearance of the secondary symptoms in this form of anaemia, such as trophic disturbances of the nails and tongue, rhagades around the corners of the mouth—all symptoms which the American authors and *Vannotti* have described as occurring in severe B_2-hypovitaminosis and in pellagra and which vanish under suitable vitamin treatment. Our attention having been drawn to this matter, we ourselves were frequently able to determine a definite hypovitaminosis B_2 and PP in cases of idiopathic hypochromic anaemia. Now iron, lactoflavin and nicotinic acid are closely interconnected in the regulation of cellular respiration, namely, in the form of catalysts (iron-containing enzymes of respiration, yellow enzyme of respiration and nicotinic acid dehydrase). The fact that the lack of iron or even of the above-mentioned vitamins provokes similar symptoms would appear to indicate that the same pathological tissue reaction may be caused by insufficiency of one or the other, or of both, the biological catalysts. Achlorhydria would not be accounted for by a casual gastritis, but by a fundamental change of balance in cellular respiration in which (according to the theory of *Jung* and of certain American authors) the disturbances of the system of cell respiration would impede the supply of the H-ions needed for hydrochloric acid synthesis.

This condition would therefore represent a profound disturbance of the general metabolism, in the aetiology of which a preponderant role would be played by a deficiency in the biologically active separable iron fraction; in addition to anaemia of the circu-

lation it might also provoke a tissue anaemia which in its turn would be closely interconnected with a disturbance of cellular chemical processes and of the co-operating biologically active substances (haemins and vitamins).

Finally, certain anaemias with a diminished serum iron content are produced by the combined interaction of numerous other aetiological factors.

The anaemias showing a normal or increased serum iron content are less numerous, although they are of considerable interest in the consideration of iron metabolism.

Certain forms of hypochromic anaemia with a normal iron metabolism, while not reacting to iron treatment, do so to parenteral administration of pyrrol bodies contained in certain amino acids (*Fontès, Thivolle, Whipple*). These forms do not represent a lack of iron, but of chromogen, that is, the pyrrol nucleus indispensable for the formation of porphyrin for the blood pigment. In the aregenerative anaemias a functional inertia of the bone-marrow impedes the synthesis of haemoglobin without the metabolism of iron being involved.

Finally, prior to liver treatment, pernicious anaemia regularly shows a marked increase of the A- and B-irons, i.e. the iron of the separable complexes. Here then, we are faced by a condition similar to that described in the case of haemolysis. Indeed, in pernicious anaemia the haemolytic components are very important factors. But an exact analysis of our four iron fractions shows us that the C and D, that is the iron of the non-separable complexes and of the protein precipitate, are greatly diminished in pernicious anaemia (which is not the case in haemolysis), although the total serum iron often rises to nearly normal values. This reduction is in part due to the hypo-proteinaemia, in part to the fundamentally different pathogenesis of the two forms of anaemia. Indeed, pernicious anaemia represents a fundamental disturbance of the erythropoiesis during which, as indicated above, the bone-marrow reverts to an early-embryonic state of functioning. By means of increased blood decomposition and an insufficient utilisation of the iron by the erythroblasts, the separable iron in the blood and tissues becomes increased, resulting in the formation of a porphyrin (iron-free haemoglobin). At this point we wish to draw a parallel between pernicious anaemia and porphyria. In both conditions we note the appearance in increased quantities of porphyrin in the blood and tissues (representing the synthesis of blood: porphyrin I —independent of blood porphyrin—porphyrin III).

In porphyria there would therefore be a disturbance in the formation of tissue haemin, as in the formation of blood haemins in the bone-marrow in pernicious anaemia.

If a case of pernicious anaemia is treated with liver extracts and a reticulocyte crisis occurs, there is a complete change of the serum iron content, both quantitative and qualitative. The value of the A-iron, or easily separable fraction, rapidly declines. The same thing occurs in the case of the not easily separable B-iron if the erythropoietic reaction is intensive. A few weeks later, if the liver treatment is continued, the anaemia and general condition will improve, whilst the A- and B-irons tend to stabilise at rather lower values. From then on the C- and D-irons begin progressively to augment. The regeneration of the bone-marrow appears to be based in the main on co-operation of the separable iron. The C-iron appears to be indirectly bound up with the erythropoiesis, for once the metabolism of the separable iron has been stabilised this iron fraction also strives to attain its normal value. Thus the C and D fractions would, at least in part, represent the final product of a further working up of the separable iron. Finally, the value of the iron of the protein precipitate becomes stabilised when the proteins of the plasma start increasing, once the permanent improvement of the anaemia is assured.

These same fluctuations of the various iron fractions are found in practically all forms of increased haematopoiesis, whether this be at the time of blood regeneration after a severe anaemia, or in the various types of bone-marrow hyper-functioning (polycythemia), or in our experiments *in vitro* in which we were able to follow the iron metabolism in the isolated bone-marrow under the influence of artificial stimulation of its activity.

In pernicious anaemia (see above) iron metabolism is disturbed by the fact that the erythroblast, which has reverted to an embryonal state of functioning, is no longer able to synthesise the haemoglobin (formation of parphyrin I, which cannot be combined with iron). The blood cell receives haemoglobin and porphyrin simultaneously. In hypochromic anaemia, on the other hand, it is a lack of iron or possibly some other synthetic substance of the chromogen, which paralyses haemoglobin formation. In the introduction of iron into the bone-marrow the reticulo-endothelial system plays a very important part.

Iron and the reticulo-endothelial system. The study of iron metabolism in connection with the functioning of the reticulum, as observed by means of blocking or stimulation of the reticulum in animal experiments, has led to the following conclusions: The reticulum manifests in the face of the blocking certain functional differences, dependent upon whether it is located at the periphery (liver, spleen) or in the centre of the erythropoietic system (bone-marrow). The blocking takes place more rapidly at the periphery than in the bone-marrow. There exists between the various sections

of this system a functional difference and a temporal dissociation in relation to the reaction to the blocking—a fact that would explain that the reticulum of the bone-marrow manifests less intensive activity in blood destruction that the reticulum of the organs of haemolysis (liver and spleen). On the other hand, the bone-marrow would exercise selection in taking up the non-haemoglobin iron and the pigments needed for the formation of the porphyrin ring. This serves to explain the various reactions of the serum iron to a stimulus or to an inhibition of the reticulum, as provoked by blocking. This functional co-operation of the reticulum and erythroblasts is particularly noticeable in lead poisoning; for a study of iron metabolism has shown that lead prevents iron from being utilised for the synthesis of haemoglobin in the erythroblasts, without in any way influencing the delivery of chromogen. This leads to the formation of an iron-free haemoglobin, that is, porphyrin. The lead does not affect the bone-marrow if the reticulum is blocked prior to lead intoxication. The lead has an effect on the formation of the haemoglobin only, but not on the other " haems ". In fact, in lead poisoning, we observe an anaemia, but the cytochrome of the tissues does not decrease; it can even increase.

This fact shows that the mechanism and the place of synthesis of the " haem " are different according to the nature and the function of the corresponding pigment. On the other hand, haemoglobin formation depends upon the functional co-operation of the reticulum of the organs of blood decomposition, which prepare the iron for blood formation, with the reticulum of the bone-marrow, which retains and stores the iron and at the appropriate time delivers it to the erythroblast.

In relation to this essential activity of the marrow in iron metabolism, we see that all the iron metabolism converges to the formation of haemoglobin. Thus, we can explain the very important increase of the iron absorption in cases of anaemia and the rapidity with which the organism is able to utilise the iron for the synthesis of the haemoglobin. All these facts, observed with the help of radioactive iron by *Whipple* and his collaborators, are of extreme importance for understanding the regulation of iron metabolism; this regulation takes place above all according to the requirements of erythropoiesis and, to a lesser extent, according to the caloric and catabolic needs of the tissues.

The mechanism of iron absorption is then the most important regulator of iron metabolism (ferritin system). On the other hand, iron excretion is only a secondary fact; however, this can play an important part in cases where iron suddenly increases in the circulation (injection of iron or intense haemolysis). In these

cases, the kidney reacts rapidly and excretes the iron; thus it prevents non-haemoglobin iron exceeding a certain level.

Iron Metabolism and Liver Activity. In the course of our exposition of iron metabolism we have repeatedly mentioned the relations existing between iron and hepatic activity. Hitherto we have considered the liver as an organ for the storage and conversion of iron, especially during haemolysis, a characteristic mainly based on its reticulo-endothelial system. It now remains for us to consider the liver as an organ of iron excretion.

Hemmeler and other authors have determined a great increase of the serum iron content in catarrhal icterus, which *Vannotti* recently also established in hepatitis epidemica (infectious icterus). This fact is interpreted by *Hemmeler* as a symptom of defective iron excretion by the liver. But we have seen above that the organism's capacity to excrete iron is small, and that the balance of iron is principally maintained by selective absorption. An exact analysis of the iron fractions in the course of simple icterus shows that there is an increase of A- and B-iron, as we have previously observed in haemolysis and pernicious anaemia. Moreover, in haemolysis the values of the C- and D-iron are normal or increased, whilst in pernicious anaemia they usually show a greatly diminished value. The view that in infectious icterus there is defective excretion of iron through the liver does not strike us as very plausible, because in obstructive icterus in which bile excretion is arrested there is no increase of serum iron. On the other hand, we know that the deposition of iron in the liver depends upon the functioning of the hepatic parenchyma and upon the condition of the general metabolism. Injury to the hepatic cell, even if it can be remedied, may considerably impede the deposition of the circulating iron in the hepatic parenchyma. The separable iron, which is no longer retained by the liver, accumulates in the blood. This hypothesis has been confirmed experimentally. If the hepatic parenchyma is poisoned with phosphorus it is no longer capable of retaining iron which is administered parenterally.

We have pursued further our study of the role of the liver in iron excretion. Observations in animal experiments have shown us that there probably exists a double entero-hepatic circulation for iron, such as is known to be the case for urobilin. The biliary iron appears to be reabsorbed through the intestine and to be returned to the liver via the portal vein. The object of this mechanism would appear to be to convert the physico-chemical condition of the iron which has been excreted through the bile and rendered practically useless for the organism and to render a portion of the biliary iron biologically active again. This would explain the occurrence of anaemia after biliary fistulas.

Thus it is seen that the liver plays an exceedingly important role in iron metabolism, both as an organ of storage and one of conversion and excretion. Iron intake is not necessarily accompanied by an increase of the bile iron. But the latter can be seen if the hepatic cell is injured, that is, if it is unable any longer to retain the iron. Finally, we frequently find a rise of the biliary iron values in increased haemolysis. Moreover, attention should be drawn to the fact that a study of iron metabolism in haemochromatosis has revealed a disturbance of the functional co-operation between the Kupffer's cell and the hepatic cell. Thus it appears that the passage of the iron of the reticulum to the hepatic cell would be impeded, leading to iron retention in the blood and tissues.

Iron and General Metabolism. We have repeatedly emphasised the importance of iron as a biological catalyst, that is, as a substance indispensable for the regulation of the cellular chemical exchanges, particularly in connection with tissue respiration. It is therefore highly probable that general metabolism and, especially, basal metabolism exert a certain influence on iron metabolism upon which they are either directly or indirectly dependent. The quantities of iron which partake actively in the chemical processes of the tissues are comparatively small. This fact adds considerable difficulty to the task of searching out this special iron fraction under normal conditions.

Nevertheless we have been able to study the physiological fluctuations of the serum iron level in the course of great muscular exertion, particularly in the mountains, where the respiratory exchanges are often greatly affected owing to the reduction of the partial pressure of oxygen.

At great altitudes, due to increased physiological haemolysis, there is at first a reduction in the number of red blood corpuscles, leading to a rise of the bilirubin and serum iron level. During a prolonged sojourn in the mountains it is therefore possible to note an augmented iron value, particularly of the active iron (separable iron), and this is gradually followed by increased blood formation. The haemolysis becomes intensified, chiefly as a result of bodily exertions; but if the muscular effort is exceedingly great and of long duration there is a temporary diminution of the iron value, which may be particularly pronounced in cases of complete exhaustion. The physiological haemolysis observed in the mountains would therefore represent a compensatory mechanism of great biological value. It would permit the liberation of haemoglobin iron, partly for the purpose of converting it into the biologically active iron indispensable to meet the increased needs of respiration and of the cellular chemical processes. We have also

been able to make similar observations in the plains or at moderate altitudes in connection with particularly strenuous sporting activities. During muscular exertion the organism mobilises its reserves of separable iron and the A- and B-iron greatly diminish. But if the organism has not at its disposal a sufficient quantity of iron a compensatory haemolysis will take place, followed by a rise in iron.

A similar phenomenon would also occur in certain pathological forms of very active metabolism, as in fever; where the fall in the iron, particularly the separable iron, should be partly attributed to a mobilisation of this metal in the reticulo-endothelial system, and in part to increased need of the tissues in respiration.

This intermediate dependence of the serum iron level upon the changes in general metabolism can be found again in another domain, i.e., in severe conditions of cyanosis of various origins. Actually, in cyanosis, a definite rise of the serum iron content can be noted, affecting chiefly the separable iron, particularly if this cyanosis is to be attributed to an insufficiency of the pulmonary circulation (insufficiency of the right side of the heart, pulmonary stasis in mitral stenosis, etc.). It is not so marked when the cyanosis is caused by a reduced surface of respiration. This increase is not necessarily to be ascribed to augmented haemolysis; frequently it is a transient phenomenon occurring at the time of the severest clinical symptoms.

In addition we have studied iron metabolism in connection with the special form of cyanosis observed as a result of sulphanilamide treatment. It would appear that this cyanosis is caused by the appearance of methaemoglobin. The initiation of sulphanilamide treatment is followed by a great increase of the separable iron, which is not necessarily dependent upon excessive blood destruction. Neither the serum bilirubin nor the urobilin need be augmented, but we regularly observe great porphyrin increase. Similar results are noted from an exact analysis in yeast cultures of the various enzymes and pigments involved in cell respiration. The sulphonamides produce a definite reduction of oxydase, catalase and cytochrome, and an increase of the separable iron in yeast, during which the content of vitamins B_1 and B_2 remains practically unchanged. All these investigations have led us to assume that the sulphonamides lead to a certain degree of disturbance of the iron-porphyrin complexes which participate in cellular respiration, which is clinically revealed by a liberation of iron and porphyrin. These findings would therefore lend support to the views of those authors who consider that one of the effects (or a secondary effect) of the sulphonamides is to cause a disturbance of the system of cell respiration injurious to microbes.

It remains for us to consider the changes undergone by the serum iron content in disturbances of thyroid functioning. In this case one single iron determination does not enable us to determine the effect of thyroid on the serum iron and this is why no positive results can be obtained by a number of individual observations. If, however, the curves of the serum iron and its fractions are followed while under the influence of an inhibition or stimulation of the thyroid activity, it is often possible to determine close functional relations between the latter and the iron.

Hypothyroidism is usually accompanied by a reduced serum iron content, proceeding in part from faulty iron absorption due to achlorhydria, and probably in part from a diminished need of the tissue respiration—a phenomenon that is characteristic of a reduction of the basal metabolism. Thyroid treatment frequently provokes a transient mobilisation of iron, but the rise in the serum iron content is rapidly neutralised by an augmented consumption of iron in the periphery.

The study of iron metabolism in hypothyroidism furnishes us indirectly with an explanation of the frequent appearance of anaemia in myxoedema. It is no mere accident that the reduction of the thyroid activity is accompanied by anaemia and that the latter sometimes assumes a pernicious form. The lack of iron in hypothyroidism is probably manifested not only in the tissues, but also in the bone-marrow, whereby it might impede erythropoiesis. Thus "circulatory anaemia" would be associated with "tissue anaemia". With regard to the development of hypothyroidism in the course of pernicious anaemia it may be stated that this might be explained by a compensatory reaction of the thyroid toward the activation of cell oxidation released by the enrichment of the tissues in separable active iron, characteristic of this form of anaemia. This view would account for the fact that in pernicious anaemia there is frequently a rise of the basal metabolism and increase of the serum iron.

These observations, although still confined to the domain of hypothesis, offer an insight into the functional and regulatory relations existing between iron metabolism and the thyroid. By this means it might also be possible to partially explain the role played by the thyroid in erythropoiesis.

The clinical observation of a case of exophthalmic goitre accompanied by severe iron-deficiency anaemia, in which the basal metabolism only showed a typical rise with an exacerbation of the general condition as a result of intensive iron treatment, recently furnished us with a clinical confirmation of the above-mentioned concept regarding the close functional co-operation between iron (especially tissue iron) and the thyroid.

These few considerations suffice to show us the practical importance of exact investigation of iron metabolism by means of the non-haemoglobin iron contained in the serum. It not only offers an insight into the iron balance (absorption and excretion), but also, and more especially, into the complex process of the blood change (blood formation and destruction); furthermore, into the important role played by the liver in intermediary iron metabolism; and finally, into certain regulatory mechanisms of the cellular chemical processes, above all of cell oxidation (muscular activity), which are the concrete manifestation of the energy exchanges of the whole body.

General Considerations

Having reached the end of our exposition, we should like to suggest a few hypothetical problems as a basis for research connected with iron metabolism. The study of iron metabolism has hitherto been chiefly confined to the problem of the formation of blood pigment, with the result that the clinical significance of this metal was practically confined to its recognition in the treatment of anaemia. But we have seen that iron plays a role at least equally important, if not more so, as a regulator of the cellular chemical processes and as a constituent of the cytoplasm. Hence severe iron deficiency might prove very dangerous for the normal functioning of the tissues. In order to prevent the danger of iron-impoverishment of the tissues, which might have the gravest consequences, the organism possesses the power of exhausting its iron reserves and of greatly reducing its haemoglobin formation, without the biologically active iron of the tissues being perceptibly diminished.

It is certain that the most important variations in iron metabolism are in relation to the formation of haemoglobin, which requires great quantities of iron. However, the needs of the tissues for iron are not negligible, although the exchanges, which are slower, do not provoke sudden variations in iron metabolism. These considerations are supported by the observations made by *Vannotti* and his collaborators on the adaptation of haemic pigments to oxygen lack at high altitudes. In fact, we see that at 6,000–7,000 m. the organism mobilises great quantities of iron in order to increase the percentage of its "haem" pigments. But while the haemoglobin shows an increase of 30%, the myoglobin increases by 50–70% and the cytochrome C by 100–150%.

The presence, distribution and activity of these pigments are responsible for the regulation and even the nature of the most important biochemical processes of the organism. The origin of these substances is based on the metabolism of the porphyrin ring

and of iron. Thus clinical science must not confine itself to following the normal and pathological metabolism of the blood haemin, i.e. the haemoglobin, in all its phases, but must also take into account the changes occurring in the tissue haemins.

The following design of normal iron metabolism

Haemoglobin (Blood haemin) ⟵ Circulating iron ⟶ Cell haems

must correspond to the following design of iron metabolism in pathology:

Disturbance of haemoglobin formation ⟵ Disturbances of iron metabolism ⟶ Disturbances of functions of cell haems

Within this framework we can already consider a disturbance of these regulations as follows:

Circulatory anaemia ⟵ Lack of iron or arrest of iron function ⟶ Tissue anaemia (more rare)

A number of regulations and biological mechanisms complete this design.

Under certain pathological conditions there is formed a haemoglobin without iron, i.e. a porphyrin. This occurs when the functioning of the cell is disturbed and the iron can no longer enter the porphyrin ring (lead poisoning); in other cases it is not the metal which is directly affected, but the chromogen, in which case the organism becomes the site of a pathological porphyrin synthesis with the formation of a porphyrin (Porphrin I) differing isomerically from normal blood pigment (Porphyrin III). In such a case the iron cannot participate in the formation of a Haemoglobin I, which would differ from the physiological Haemoglobin III by isomerism. Thus a change in the iron metabolism is reinforced by a disturbance of the pigment metabolism.

By means of its biological activity the cell iron enters into close relationship with a great number of other catalysts, which in their turn are functionally bound up with certain vitamins (lactoflavin, aneurin, nicotinic acid and ascorbic acid). Indeed, there exists co-operation between the numerous iron-containing enzymes and pigments of the cell and *Warburg's* respiratory yellow enzyme (B_2), carboxylase (B_1) and certain redox systems (C). It is therefore not accidental that the clinical picture of a disturbance in iron metabolism as seen, for instance, in essential hypochromic anaemia with its blood and tissue changes, may be reinforced by certain symptoms proper to disturbances of vitamin metabolism, and that the supply of certain vitamins, as we have previously stressed, may

affect both the metabolism of the porphyrin ring and the iron metabolism.

The function of the tissue iron, as a regulator of cellular oxidation, is further connected with the mechanism of total respiration, that is, with the basal metabolism, which in its turn is influenced by the endocrine system and especially by the thyroid. Thus there must exist certain relationships between iron and thyroid.

As a matter of fact, we were able to determine that the disturbances of the thyroid functioning are able to influence iron metabolism, and that, conversely, a disturbance of the iron metabolism indirectly affects the thyroid functioning. Thus iron metabolism is associated with the activity of the thyroid through its participation in the regulation of the tissue chemical processes. In this way it might also be possible to explain the indirect influence of the thyroid on blood formation, a process which is known to be particularly dependent upon iron metabolism. This would serve to explain the connection existing in human pathology between disturbances of the thyroid and the various forms of anaemia.

Certain connections clinically established between the thyroid and bone-marrow which hitherto have found no satisfactory aetiological explanation (as for instance, anaemia and hypothyroidism) would thereby be rendered intelligible.

One additional question will be considered. Whether *Barkan's*, *Heilmeyer's*, or our own method of determination is used, it is almost exclusively the fraction of the separable iron, i.e. the iron taken up by hydrochloric acid, that furnishes the most interesting values for the study of total iron metabolism. This iron, which is more or less firmly bound to the complexes but which is still separable, can be partly identified with the active iron of *Starkenstein* and with the freshly absorbed iron of *Whipple*. In the course of haemolysis the value of this iron increases and it diminishes during intensive blood regeneration. It rises again during the fundamental erythropoietic changes occurring in pernicious anaemia and in certain hepatic disturbances. It sinks as a result of the influence of special toxi-infectious processes, a rise of the basal metabolism, etc.

Although the methods of *Heilmeyer* and of the B-fraction do not cover the total serum iron, by this means it is possible to determine the fraction of most interest and importance for the study of normal and pathological serum iron metabolism.

But not until the fluctuations of the four fractions of the total iron have been separately studied is it possible to acquire a clearer and more complete insight into normal and pathological iron metabolism.

Thus, in addition to the disturbances which effect a reduction or an increase of the total serum iron, we can, as we have seen

above, observe certain pathological conditions in which only one or two fractions show any change and which are neutralised by opposite variations of the remaining fractions. In certain cases, therefore, on the basis of a simple determination of the fractions A and B, or of the *Barkan* or *Heilmeyer* iron, it would have been possible to believe that there was a diminished serum iron content; but the determination of the four fractions and of the total iron revealed a normal or even increased serum iron content.

Hence in pathology the normal distribution between the various fractions may often be disturbed without the value of the " total iron " being greatly altered. The non-separable fraction may fall considerably, in favour of the separable fraction, and *vice versa.* If we are confronted by a great reduction of A- and B-iron (the separable iron), offset by an increase of the non-separable iron and of the iron of the protein precipitate, we cannot immediately speak of a reduced serum iron level or iron deficiency. But in such cases the organism is endangered, since it lacks just that iron fraction which is biologically most important for the normal functioning of blood and tissue. A shifting of the normal distribution of the iron fractions, unaccompanied by a genuine quantitative iron deficiency, may thus provoke severe forms of anaemia or functional disturbances of the tissues. Although the body is supplied with adequate amounts of circulating iron, it may nevertheless present a form of anaemia which it would be erroneous to designate as iron-deficiency anaemia. On the other hand, it is possible that there exists, together with a normal or increased value of the separable iron, a great reduction of the non-separable iron, which might lead to a normal or even diminished total iron content in the serum. The pathological shifting of the various fractions is therefore often the expression of defective iron utilisation, which may be clinically manifested in the form of anaemia or by other symptoms.

Under such pathological conditions we must direct all our efforts towards elucidating the cause of this quantitative change in the distribution of the serum iron, and seek a method of restoring the qualitative equilibrium between the fractions.

We are still in the realm of hypothesis, but, thanks to a great number of clinical observations which we have assembled in the course of our investigations, we are encouraged to expect definite data regarding the mechanism whereby certain therapeutic successes have been attained, and the possibility of further therapeutic progress in the domain of iron metabolism.

This fact should be particularly stressed. In these cases the effect of the liver extracts is very probably connected with the influence exerted by the liver on iron metabolism. The liver plays

a special role as an organ not only for the storage, but also for the conversion, of iron; this it does both through the activity (enzymatic in nature) of its parenchyma and through the entero-hepatic iron circulation. This iron is excreted through the bile as a biologically useless substance, is in part newly elaborated in the intestine, and is finally returned again to the liver through the portal vein. Thus, regarded from two points of view, the liver is seen to be a highly important agent in the distribution of the various iron fractions throughout the organism. It is obvious that by stimulating the hepatic function and with the co-operation of enzymes the liver extracts may exert a special influence on the regulation of the non-haemoglobin iron and its fractions.

These considerations, furthermore, lead us to assume that the stimulation of bile formation and excretion leads to increased iron excretion, that is, to the stimulation of the entero-hepatic circulation of this metal, and hence to its activation for biological ends. It is, therefore, by no means excluded that by employing methods to stimulate the production and excretion of bile we might succeed in changing the iron fractions in favour of the separable iron.

The influence of the thyroid on the tissue iron and indirectly on erythropoiesis, and finally the distribution of the iron fractions in the sense of increasing the separable iron, lead us to expect that in the treatment of certain forms of anaemia there will be a greater application of thyroid extracts, possibly in combination with extracts of the liver and cholagogues, in addition to iron. The functional collaboration of iron and certain vitamins in cellular catalysis renders it probable that a useful therapeutic co-operation of iron and vitamins B_1, B_2, PP and C will be brought about (the latter of which probably reinforce the effect of the biologically active metal).

These few therapeutic considerations are offered primarily as suggestions for investigation. They show that the study of iron metabolism by the fractional determinations of serum iron is not merely of theoretical interest, but offers additional indications regarding the diagnosis, prognosis and treatment of many pathological conditions.

As a matter of fact, more detailed acquaintance with certain mechanisms, which have not yet been sufficiently examined, permits us to enlarge our concepts, which hitherto have been confined to the activity of iron in blood formation. Thus iron is assured a position of clinical importance as a biologically indispensable element, responsible in the general metabolism of the organism, in tissue and pigment formation, for the cellular catalysis and the control of countless important reactions.

Before ending Part Two, it seems to us indispensable to bring to the attention of the reader the important work of *Pauling* and his collaborators on the molecular structure of derivatives of ferri- and ferroporphyrins. This work enables the chemical constitution and the physical structure of animal iron containing pigments to be explained. An excellent summary of this question is to be found in the article by *Drabkin*.

PART THREE

CLINICAL INDICATIONS AND PRACTICAL CONSIDERATIONS IN IRON THERAPY

After having discussed at length the principal problems connected with the iron metabolism of the human organism we can now pass to the practical consideration of iron treatment.

It is necessary that we bear in mind the following important points in connection with iron therapy.

In most conditions of iron deficiency the organism shows an increased capacity of iron absorption through the intestine. But this absorption will only attain therapeutic dimensions if sufficiently large quantities of iron and easily absorbed iron compounds are administered. Furthermore, the normal gastro-intestinal activity must not be disturbed. A lack of hydrochloric acid in the stomach may impede the ionisation of the iron and make it impossible for absorption to take place. Severe intestinal disturbances to the mucous membrane may prevent the iron administered *per os* from being utilised.

As a general rule the food contains insufficient amounts of iron that can be utilised. It is not the total quantity of iron but the amount that can be liberated from the gastric juice that is of importance for the organism. *Heilmeyer* and *Mutius* give the following iron values:

Liver (raw)	1228 γ%	Potato (raw)	112 γ%
Chicken (cooked)	169 γ%	Sauerkraut (raw)	137 γ%
Potato (cooked)	32 γ%	Spinach (raw)	294 γ%
Asparagus (cooked)	48 γ%	Tomato (raw)	99 γ%
Spinach (cooked)	88 γ%	Bread (according to kind)	76–245 γ%
Apple (raw)	68 γ%		
Rice	11 γ%	Noodles	14 γ%
Cow's milk	15 γ%	Human milk	18 γ%
Yolk of egg	96 γ%	White of egg	26 γ%
Butter	48 γ%	Cheese	19 γ%
Pork (raw)	124 γ%		

These figures are important, since in certain cases, as a result of an appropriate diet rich in iron, it is often possible to combat successfully chronic conditions of iron deficiency. This is particularly important for women during pregnancy and lactation, as well as for the child.

Active iron therapy necessitates great quantities of iron which are readily absorbed. If oral treatment fails, chiefly due to inadequate iron absorption, the iron may be administered paren-

terally and more especially intravenously. This type of iron therapy is frequently insufficient, since the quantities of iron administered in this way are too small (a few milligrams).

Furthermore, it is important to note that the iron treatment is necessary to combat not only the various forms of anaemia accompanied by iron deficiency, but also the numerous clinical symptoms of iron deficiency, which are not necessarily associated with anaemia. This is particularly the case in chronic conditions of infection, during protracted febrile periods, convalescence, pregnancy, etc. In such cases the condition is usually one of a lack of the iron needed for the building up of the body and to meet the catalytic needs of the tissues.

The Most Important Iron Preparations

In accordance with the statements contained in Part I of this book relative to the physiological significance of divalent and trivalent iron in connection with absorption and its biological utilisation, preference is given to-day, from a therapeutic point of view, to divalent iron preparations (ferrous), that is, to iron in a reduced form which is most easily absorbed. Metallic iron has been and is still often used, as it produces ferrous chloride in the stomach; this can readily be absorbed.

In addition to oral iron administration it has been possible in recent years to give parenteral injections of iron. This method is of particular value in cases where the iron provokes unpleasant secondary symptoms in the gastro-intestinal tract and where the intestinal absorption is impeded by functional disturbance or by inflammatory-degenerative changes in the digestive tract. The parenteral administration of iron is of great value here, not merely as a special form of iron therapy, but also as a means of specifically stimulating the activity of the bone-marrow. This is an instance of selective stimulation of erythropoiesis which is manifested by an Hb. increase which is greater than the Hb. quantities which might have been produced by the injected iron. But the present-day importance of parenteral iron treatment by means of stabilised divalent iron preparations is based on the fact that this form of iron plays an important role in the biocatalytic processes in the tissues.

Starkenstein, who studied in detail the problem of the modifications undergone by iron after intravenous injection, found that ferrous chloride is rapidly oxidised in the blood and is transformed into ferri-globulin complexes. He states that it is in its bivalent form that upon reaching the tissues the iron exercises its function of a biological catalyst. Once oxidised it is again transformed

into bivalent iron, so that it may be incorporated in the molecule of haemoglobin or enrich the storage iron.

He considers, on the other hand, that the ferric salts are immediately excreted or rapidly transported to the organs of storage. The findings of *Starkenstein* would thus lead to the assumption that the only therapeutic parenteral form would be the bivalent form. *Fleischaker* and *Schürer-Waldheim* tested ferrous chloride and *Heilmeyer* and his followers tested the bivalent ascorbate of iron. The last-named authors found that this product possessed great therapeutic power. Ferrous gluconate was used for the first time by *Retznikoff* and *Goebel*. The trivalent salts (ferric) were also successfully used. It was principally by the use of ferric cacodylate that *Lederer* obtained interesting therapeutic results. *Starkenstein's* view that only the ferrous salts possess therapeutic action would thus appear to lack confirmation. It should, moreover, be stressed that the intravenous injection of ferrous salts is often followed by toxic manifestations or by unpleasant vascular reactions.

Ascorbate of iron must be injected in small doses and very slowly, since it frequently provokes a violent vaso-dilatation in the skin. Ferric cacodylate is usually better tolerated (although it may provoke nausea and gastric disturbances).

However, two points of interest should be stressed in connection with the parenteral iron treatment. In cases where the latter had a favourable effect on the anaemia *Heilmeyer* and *Plötner* observed that the increase of haemoglobin was definitely greater than the amount of iron injected. This accordingly led these authors to believe that the iron served to stimulate haematopoiesis.

On the other hand, the clinical results obtained by intravenous injections of iron were often extremely inadequate, above all in the treatment of idiopathic hypochromic anaemia in which, even after a prolonged series of injections, it is practically impossible either to obtain an improvement in the blood counts or to observe the action of erythropoietic stimulation as envisaged by *Heilmeyer* and *Plötner*. This observation, which we have repeatedly made and which in part corresponds to similar observations by *Büchmann* and *Kohler* and by *Fowler* and *Barer*, would coincide with the experimental results obtained by *Lintzel* in the case of rats. After a prolonged parenteral administration of iron, under various experimental conditions, the last-named failed to find any augmentation of the haemoglobin content.

In numerous cases treated by ourselves, amounting to approximately a hundred intravenous injections of 6 mg. of iron, the effect on the anaemia was sometimes negative, the blood count showing no appreciable change. Very often, on the other hand, the

subjective condition of the patient was greatly improved. His weight increased, the secondary symptoms of idiopathic hypochromic anaemia (lethargy, fatigue, rhagades around the lips, lesions of the mucosa, etc.) definitely diminished, as a result of which it was possible to observe a definite improvement of the general condition, unaccompanied by any amelioration of the blood picture.

It would therefore appear that the influence of iron injected intravenously is generally favourable for tissue metabolism in general, although often only inadequate for the erythropoiesis. This may be due to the fact that an insufficient amount of iron is supplied to the organism by intravenous injection. As a matter of fact, as has been correctly emphasised by *Büchmann* and *Kohler*, the quantity of iron contained in the total haemoglobin of the organism is around 2.5 g., whilst the amount of iron injected is only a few milligrams.

Nevertheless, there is one point which should not be overlooked, namely, that the intravenous administration of iron exerts a favourable therapeutic action in the sense of effecting a definite amelioration of the tissue symptoms of the hyposideraemia. Hence it must be admitted that this iron acts above all as a biological catalyst in the cells and that this mechanism receives priority over that of erythropoiesis.

For a better comprehension of this phenomenon we made use of the curve of serum iron values after intravenous administration. This curve shows a very rapid increase of serum iron immediately after injection—a rise lasting usually half an hour (rarely longer), diminishing more slowly and becoming definitely stabilised four to six hours after injection.

We observed that these curves may vary considerably in different patients and in accordance with the iron product used. Thus we noted a distinct difference between the four curves obtained after intravenous administration of the same quantity of iron, given in the form of ferrous ascorbate, ferrous lactobionate (Ferro-Calcium-Sandoz) and of ferric cacodylate or ferric chloride. As a matter of fact, the serum iron curve after intravenous injection of Sandoz iron is, with few exceptions, always definitely higher than the curve obtained in the same individual as a result of intravenous injection of the same quantities of iron in the form of ascorbate of iron. These two curves have a tendency to decline very rapidly after having attained their maximum half an hour after injection (see Diagram 2, p. 21).

The curves obtained with ferric cacodylate and ferric chloride are as high as, and sometimes even higher than, those obtained with Sandoz iron, but their form is quite different. The hypersideraemia which is provoked persists two to three hours after the injection,

when the curves of the bivalent iron tend to return to their level prior to injection. It would therefore appear that ferrous salts upon being injected intravenously are rapidly utilised, whilst the ferric salts remain in circulation for a longer time.

We can thus conclude that the ferrous salts exercise a selective action on the tissues, above all on the muscles. They operate as cellular catalysts and stimulate the synthesis of the cellular enzymes with an iron base. They are also deposited in the liver and spleen and are less extensively used at the level of the bone-marrow than in the peripheral tissues. The ferric salts, on the other hand, do not exercise any appreciable biocatalytic action at the periphery, and after having circulated in the blood for a longer time than the ferrous salts they show a preference for being deposited in the spleen and in the organs of storage, thus indirectly exerting a more pronounced influence on the erythropoiesis than do the ferrous salts. The action of the *p*-aminobenzoic acid in fixing the iron in the tissues can accordingly only operate slightly with this form of iron.

It remains to explain the differences in the serum iron curves after the injection of ascorbate and of ferrous lactobionate (Sandoz iron). This difference is probably due in part to the chemical difference between these two salts, in part to the fact that the Ferro-Calcium is more powerfully stabilised in its bivalent form than is ascorbate. This was shown by the analyses of the redox potential of these two forms of iron, by determination *in vitro* and after the addition of serum. If brought into contact with the oxygen of the air and oxy-haemoglobin, ascorbate of iron oxidises more easily than does the Ferro-Calcium complex.

We therefore possess to-day two groups of products based on iron for intravenous or parenteral administration, possessing different pharmacological characteristics; the one is based on stabilised ferrous iron, the other on ferric iron.

The products constituted by bivalent iron (ferrous salts) would above all possess a tissue action, since they are partially fixed by the peripheral cell, where they favour the biocatalytic processes and produce an increase of pigments and cellular enzymes based on iron, chiefly at the level of the muscular fibre. This iron also passes into the service of the bone-marrow, but its haematopoietic action is thought to be comparatively limited.

The products with a trivalent iron base (ferric salts) would possess above all a haematopoietic action, becoming more readily fixed at the level of the reticulo-endothelial system, above all of the spleen, as a result of which they would participate in a particularly active manner in the metabolism of the blood pigment.

The haematopoietic action of iron administered parenterally is

necessarily limited, owing to the fact that the quantities of iron injected are small (a few mg. per injection); but usually it exercises a stimulative action on the haematopoiesis, an action which, in our opinion, is due in part to the stimulation of the reticulo-endothelial system, for which the iron possesses a certain tropism.

The administration of iron by injection is often necessary in cases in which the anaemia is accompanied by disturbances of intestinal absorption.

The tissue action of iron administered parenterally is particularly favourable in cases where there is such a lack of iron that its biocatalytic action is insufficient and is accompanied by tissue disturbances which are often of a serious nature. The parenteral administration of iron rapidly ameliorates this situation, whilst if given by mouth the results are often inadequate. By reducing the needs of the tissues in biologically active iron it can often be seen that the iron absorbed by the intestine is utilised almost exclusively for haematopoiesis. In this mechanism we also see an explanation of the stimulating effect upon erythropoiesis, as described by *Heilmeyer*, resulting from parenteral administration of iron.

Once its tissue, above all its muscular, role is ended, this iron can still be utilised for haematopoiesis. Thus it exercises a dual effect on the cellular chemical processes and upon the bone-marrow.

We indicate below a number of iron preparations most commonly used on the Continent:

Ferrum reductum (reduced iron).
1–10 g. daily per os, according to the nature and degree of the anaemia.
Ferrum sulphuricum (divalent iron sulphate).
$FeSO_4 + 7H_2O$
Ferrum carbonicum (Blaud's pill).
Well-known iron preparation. 2–3 pills three times daily.
Tinctura Ferri-pomati (Malic acid iron tincture).
Half a teaspoonful 3 times daily.
Ferrum lacticum (divalent iron lactate).
0.2–0.3 g. three times daily.
Ferrum citricum ammoniatum (trivalent iron ammonium citrate).
0.2–0.5 g. 3 times daily.
Liquor ferri albuminati (iron albuminate solution containing 0.4% iron).
1 teaspoonful to 1 dessert spoonful 3 times daily.

Proprietary Iron Preparations

Ferrostabil (stabilised divalent iron chloride).
3–6 tablets daily.
Ferro-" Redoxon" Roche (divalent iron preparation stabilised by ascorbic acid).
Ferrous Sulphate Emplets, Parke Davis.
Ferad Tabloids, Burroughs Wellcome (ferrous sulphate).
Naferon, Parke Davis (ferric sodium citrate).
3–9 tablets daily.

Iron Preparations for Intravenous Injection

Ce-Ferro (divalent iron preparation stabilised by ascorbic acid and Cystein). Equivalent to 6 and 10 mg.
Ferro-Calcium-Sandoz (iron lactobionate) (divalent iron preparation stabilised by calcium lactobionate). Equivalent to 6 mg.
Iron cacodylate (trivalent iron in arsenic compound).
Ferrinascine Roche (trivalent iron, 20 mg. Fe).

The divalent iron preparations for intravenous administration often provoke unpleasant secondary reactions, such as excitement, disagreeable sensations of heat, above all in the face, and possibly also conditions of collapse if the injection is given too rapidly. It is therefore advisable that these preparations be injected very slowly (over a period of five minutes) and that they be diluted with 10 cc. of 10% glucose. The Ferro-Calcium-Sandoz can also be injected intramuscularly.

One other important therapeutic point must be mentioned. From our pharmacological studies we know that the ferric salts are toxic to a great extent. This applies to the ionised salts, which denature the proteins and as a result exercise a toxic effect. On the other hand, the complex-bound ferric salts are well supported in intravenous administration. Thus it can be understood that certain trivalent iron compounds, such as ferric-cacodylate and the ferric compound of carbonic acid (Ferrinascin) are well tolerated after intravenous injection. The injection is unaccompanied by any kind of reaction, which is not the case with divalent iron compounds (vasomotor disturbances). Complex-bound ferric salts can be administered intravenously in fairly large amounts (20–40 mg.), but ferrous salts in only small quantities (5–10 mg.). Since the ferric salts that can be used are bound in complexes, the biological effect of the iron is only slight. Hence this iron must first be prepared by the haematopoietic organs in order to serve the purpose of haemoglobin synthesis. The greater amounts which can be given in every injection compensate for these disadvantages, with the result that to-day we possess in 20–40 mg. of ferric preparations an excellent agent for the parenteral treatment of severe iron-resistant forms of anaemia. It is to be hoped that there will soon be available commercial mixed preparations consisting of divalent and trivalent iron.

We must finally mention one additional form of iron which has been increasingly mentioned of late in the English literature. This is Ferritin, an iron-containing proteid in the spleen. This iron protein complex entirely lacks catalytic properties, but it is believed that the iron originating in haemoglobin is converted into Ferritin in the liver and spleen. It is not excluded that this organic iron compound which, according to *Bernhart and Skegge*, is said to

effect the storage of iron in the organism, may in future play a part in iron therapy.

The Use of Iron Therapy

We now propose to give some specific indications for the use of iron therapy. These statements are based on our explanations regarding iron metabolism in pathological conditions, as contained in the Second Part of this book.

A. *The Haemorrhagic Anaemias*

Acute haemorrhagic anaemia has usually a good prognosis; it is often cured automatically in a surprising manner, provided the organism possesses a sufficiency of iron reserves. The capacity of regeneration possessed by the bone-marrow is generally good after an acute haemorrhage. But if the haemorrhage is particularly severe it is usually advisable to replace the lost blood rapidly by means of blood transfusion (possibly also with plasma injections). After such a transfusion or after the onset of a typical anaemia with symptoms of iron deficiency (lowered colour index and reduced serum iron values) or in cases of a defective tendency to regeneration on the part of the bone-marrow, resort must be had to energetic peroral iron therapy.

In *chronic haemorrhagic anaemia* it is usually necessary to administer fairly large doses of iron, as the iron reserves of the organism have often been greatly reduced in consequence of continuous bleeding. Naturally, the cause of the haemorrhage must be removed; indeed, this is often the most important condition for successful therapy. We have seen in some cases that despite intense iron treatment the anaemia did not improve, and that not until the haemorrhage stopped did the anaemic condition rapidly subside.

In chronic haemorrhage the great deficiency of iron may produce general symptoms. On the one hand, there may be atrophy of the mucous membrane, brittleness of the nails and other symptoms of iron deficiency, and on the other hand, the function of erythropoiesis by the bone-marrow may be arrested to some extent, a condition manifested in retarded maturation of the normoblasts. In such cases it is often possible to observe the stimulating effect of the iron on erythropoiesis in the bone-marrow. The initiation of oral, or better still of parenteral, iron treatment greatly stimulates bone-marrow renegeration, with resulting improvement of the anaemia, which does not correspond to the quantities of iron administered. Hence it must be assumed that the iron has specifically stimulated the erythropoiesis. In

such cases large quantities of iron are needed, 5–8 g. of ferrum reductum or stabilised ferrous iron preparations, amounting to 0.3–0.5 g. of iron per day.

In these cases the parenteral and intravenous administration of iron is especially indicated owing to its stimulating effect. Finally, care must be taken to ensure a diet rich in meat protein.

B. *Alimentary Iron-deficiency Anaemia and Anaemia Accompanying Gastro-Intestinal Disturbances*

This condition is usually rapidly improved and cured as a result of iron therapy. Frequently, however, this form of anaemia is accompanied by symptoms of other manifestations of deficiency, so that in some cases the iron treatment must be reinforced by the introduction of other important substances, such as vitamins and protein bodies. A rich meat diet is indicated.

The cause of this anaemia may also lie in a faulty utilisation of the nutrient iron. If such is the case the iron assimilation must be stimulated by the administration of hydrochloric acid and pepsin or by combating the disturbed intestinal absorption (treatment of enteritis). If there is defective oral iron utilisation, protracted parenteral iron therapy will be called for.

C. *Idiopathic Hypochromic Anaemia and Chlorosis*

These conditions are often hard to treat. As these forms are accompanied by achlorhydria it is essential that hydrochloric acid and pepsin be regularly administered. As a result of experiments in absorption the ferrous preparations stabilised by ascorbic acid (Ferroredoxon, Ce-Ferro, etc.) have been shown to be the most effective. In some cases the oral iron therapy does not agree with the organism or the iron administered cannot be absorbed. In such cases intensive parenteral iron treatment must be initiated (6 mg. Fe every other day in 20–30 injections). The combined treatment is usually to be recommended. Frequently the general symptoms will improve, but after a preliminary improvement the anaemia will remain stationary. In such cases protein must be given (rich meat diet). In addition, repeated moderate blood and plasma transfusions are particularly efficacious. Furthermore, the administration of vitamins of the B-group may be helpful.

In certain cases, however, no success is attained, despite intensive therapy.

The daily iron dosages must as a rule consist of 5–10 g. of ferrum reductum and 0.2–0.6 g. of stabilised ferrous iron. The treatment must be continued until the serum iron has attained normal values. If the treatment is interrupted prematurely this

will speedily be followed by a recurrence of the clinical symptoms and anaemia. As a general rule the iron therapy must be continued for several months, with a suspension of treatment of from two to three weeks after each month of the iron treatment.

In some cases hormonal treatment can reinforce the effect of the iron (Ovariol and thyroid preparations).

D. *Iron-Deficiency Conditions in Pernicious Anaemia*

In the course of the liver treatment it is possible that after the reticulocyte crisis there may suddenly develop a state of iron deficiency, a condition which may considerably retard the cure of the anaemia. This iron deficiency is usually revealed by a rapid decline of the serum iron level as a result of quick bone-marrow regeneration. In such cases (especially in women) after a speedy initial improvement in blood regeneration there may be a sudden arrest of progress, despite intensive treatment with liver extracts. The anaemia will lose its hyperchromic character and may become hypochromic. The picture of anaemia with its secondary phenomena can only be radically cured by prompt administration of iron. Often, therefore, the combination of liver extracts with iron and vitamins of the B-group (B_1, B_2 and PP) will be necessary in the modern treatment of pernicious anaemia. Sometimes the addition of iron can reinforce and curtail the treatment with liver extracts. As a general rule it is recommended that medium to large doses of iron be given (4–6 g. ferr. reducti or 0.2–0.4 g. of stabilised ferrous iron).

E. *Conditions of Iron Deficiency with Tissue Disturbances*

The tissue disturbances accompanying conditions of iron deficiency are admirably combated with the stabilised divalent iron preparations. The biological importance of iron in the reduced form is seen in such cases and the parenteral preparations appear to have a good effect. For this reason a more extended oral treatment with stabilised ferrous preparations is given, in the form of a series of ten iron injections (one injection every other day) if necessary after an interruption of four weeks.

Sometimes excellent results can be attained by combining the iron treatment with vitamin B preparations (Becozym).

F. *Iron Therapy in Infectious Diseases and for Conditions of Convalescence*

The stimulating effect of iron on the renegeration of the bone-marrow may be of value in combating anaemic conditions occurring during or after infectious diseases.

In infection, anaemia is frequently the result of iron deficiency caused by a mobilisation of this metal in the reticulo-endothelial system, but it may also be the consequence of toxic inhibition of the bone-marrow. In such cases iron, especially given parenterally, may produce excellent results in stimulating the bone-marrow, whereas oral iron therapy appears to yield but feeble results. We are of the opinion that in these cases the catalytic activity of the iron is an important factor in supporting the over-taxed cellular respiration and all the mechanisms of defence. The value of parenteral iron therapy must be particularly borne in mind in chronic infections (tuberculosis, osteomyelitis, chronic suppurations, empyema, abscesses, polyarthritis, colitis, etc.), or during convalescence after severe acute infections (pneumonia, sepsis, typhus, etc.) and in malaria.

Repeated modest treatments of 5–10 injections at intervals of 3–4 weeks are particularly efficacious.

The especially important role played by iron in the regulation of the cellular chemical processes makes it obvious that iron administration is to be recommended for all types of heavy and prolonged muscular exertion, especially in sport. Hence it is advisable that muscular workers and sportsmen should be assured an adequate supply of iron, either in the form of food rich in iron or of small doses of iron. For this purpose the iron vitamin-containing food products, such as Vivavit (iron vitamin-containing cocoa) and Neoviton are to be recommended.

A moderate addition of iron is also specially advisable during childhood and pregnancy (10–100 mg. of stabilised divalent iron daily) or iron-containing food preparations.

Contra-indications of Iron Treatment

These are usually acute febrile conditions and acute gastro-intestinal disturbances.

The iron may provoke dyspeptic phenomena and may induce constipation. Also, dental caries has been observed after ample and continuous administration of iron during childhood.

In animal experimentation excessive doses of divalent iron given intravenously may lead to fatty degeneration of the liver. The simultaneous administration of calcium gluconate or lactobionate will protect the animal's liver against the injurious effects of iron.

The Doses and Duration of Iron Therapy

It is difficult to prescribe exact dosages for iron, especially as this depends upon the intensity and duration of the anaemia. More-

over, as we have seen, the route of administration must depend upon the type of anaemia and the condition of the gastro-intestinal tract.

We know that iron administered orally sometimes causes gastro-intestinal disturbances. This is particularly the case in gastro-enteritis, in which the irritant action of the iron on the mucous membrane may provoke both heart-burn with exacerbation of the digestive disturbances, and also intestinal manifestations taking the form of diarrhoea or constipation, tenesmus and diffuse pains. This increase of the gastro-intestinal disturbance involves a reduction of the iron absorption through the intestine. Hence it is essential when giving iron treatment to administer only the quantities of iron absolutely needed and in a form that can be absorbed. In this connection the experiments on dogs conducted by *Hahn, Bale, Lawrence* and *Whipple* are of special interest. With the help of radio-active iron these authors showed that the percentage absorption of the metal is much higher when the quantities of iron introduced per os are small. Thus they found in dogs that the administration per os of 1.2 mg. of iron effected a 60% absorption, whilst if quantities of 115 mg. were given the absorption was only 3.2%. In dogs 20–30 mg. represents the optimal amount in iron treatment.

For human beings the optimal doses of divalent iron are 300–500 mg. per day. It is best administered 3 to 5 times daily. For ferrum reductum the daily dose should be about 1 g. In the case of trivalent, oxidised iron salts which are not easily absorbed and which to-day are less frequently prescribed, the doses should be doubled.

For the intravenous treatment of the divalent iron preparations the dosage should not exceed 10 mg. The injections must be made very slowly, in order to avoid the occurrence of vasomotor phenomena. When using the trivalent iron preparations 20–40 mg. can often be injected simultaneously; but care must be taken not to give more than 60 mg., since the superfluous iron is also rapidly eliminated by the kidneys.

The duration of the oral treatment should be at least two weeks. Often it is well to repeat the treatment after an interval of 10–15 days. In the case of chronic obstinate anaemias the treatment must frequently be drawn out to cover several weeks, with the insertion after each third week of treatment of a short break lasting ten days. It may be possible to combine the treatment with some intravenous injections. In many cases the oral iron treatment must be combined with the administration of hydrochloric acid and pepsin.

The tissue manifestations of iron deficiency, such as atrophy

of the mucous membrane, rhagades around the corners of the mouth, brittle nails, etc., can often be made to recede in a conspicuous manner soon after the onset of treatment. But it would be erroneous to suspend the iron treatment soon afterwards, since relapses frequently occur. To-day in such severe cases of iron deficiency the treatment need not be stopped until the serum iron determination again shows normal values. Indeed we can sometimes observe a favourable remission of the anaemia in cases still showing low serum iron values. If the treatment is stopped at that stage it is often noted that the anaemia will recur shortly afterwards, this being evidence of the fact that the iron deposits in the organism have not yet been replaced. In some cases it is an advantage to continue giving the patient, periodically, small doses of iron, even after the normal haemoglobin and serum iron values have been reached (for instance during one week per month).

Often in chronic anaemia it is difficult to carry out iron treatment, particularly in cases of idiopathic hypochromic anaemia. In such cases an effort must be made, through patience and by resorting to determinations of the serum iron, to supplement the iron deposits of the organism by means of oral and parenteral iron preparations. In certain cases the therapeutic effect of the iron can be further reinforced by transfusion, treatment of the gastro-intestinal tract (removal of chronic enteritis, hydrochloric acid and pepsin, etc.), the administration of protein, endocrine therapy, etc.

Certainly it is important that the physician should not immediately resort to iron treatment as soon as he has discovered that his patient has anaemia. This treatment can only be initiated once the exact nature and pathogenesis of the condition has been systematically determined and when general treatment is already being applied to combat the infirmity.

We must be prudent in the use of intravenous iron. Certain authors have injected very great quantities (above 1 g.). However, the patients may have disagreeable reactions and present serious lesions which make treatment with large quantities of intravenous iron dangerous. (See *Tompkins, Goetsch, Moor* and *Minnich.*)

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